

# IL-17a promotes sociability in mouse models of neurodevelopmental disorders

<https://doi.org/10.1038/s41586-019-1843-6>

Received: 17 December 2018

Accepted: 30 October 2019

Published online: 18 December 2019

Michael Douglas Reed<sup>1,2,7</sup>, Yeong Shin Yim<sup>1,2,7</sup>, Ralf D. Wimmer<sup>1,2,3</sup>, Hyunju Kim<sup>4</sup>, Changhyeon Ryu<sup>1,2</sup>, Gwyneth Margaret Welch<sup>1,2</sup>, Matias Andina<sup>1,2</sup>, Hunter Oren King<sup>2</sup>, Ari Waisman<sup>5</sup>, Michael M. Halassa<sup>1,2,3</sup>, Jun R. Huh<sup>4,6\*</sup> & Gloria B. Choi<sup>1,2\*</sup>

A subset of children with autism spectrum disorder appear to show an improvement in their behavioural symptoms during the course of a fever, a sign of systemic inflammation<sup>1,2</sup>. Here we elucidate the molecular and neural mechanisms that underlie the beneficial effects of inflammation on social behaviour deficits in mice. We compared an environmental model of neurodevelopmental disorders in which mice were exposed to maternal immune activation (MIA) during embryogenesis<sup>3,4</sup> with mouse models that are genetically deficient for contactin-associated protein-like 2 (*Cntnap2*)<sup>5</sup>, fragile X mental retardation-1 (*Fmr1*)<sup>6</sup> or Sh3 and multiple ankyrin repeat domains 3 (*Shank3*)<sup>7</sup>. We establish that the social behaviour deficits in offspring exposed to MIA can be temporarily rescued by the inflammatory response elicited by the administration of lipopolysaccharide (LPS). This behavioural rescue was accompanied by a reduction in neuronal activity in the primary somatosensory cortex dysgranular zone (S1DZ), the hyperactivity of which was previously implicated in the manifestation of behavioural phenotypes associated with offspring exposed to MIA<sup>8</sup>. By contrast, we did not observe an LPS-induced rescue of social deficits in the monogenic models. We demonstrate that the differences in responsiveness to the LPS treatment between the MIA and the monogenic models emerge from differences in the levels of cytokine production. LPS treatment in monogenic mutant mice did not induce amounts of interleukin-17a (IL-17a) comparable to those induced in MIA offspring; bypassing this difference by directly delivering IL-17a into S1DZ was sufficient to promote sociability in monogenic mutant mice as well as in MIA offspring. Conversely, abrogating the expression of IL-17 receptor subunit a (IL-17Ra) in the neurons of the S1DZ eliminated the ability of LPS to reverse the sociability phenotypes in MIA offspring. Our data support a neuroimmune mechanism that underlies neurodevelopmental disorders in which the production of IL-17a during inflammation can ameliorate the expression of social behaviour deficits by directly affecting neuronal activity in the central nervous system.

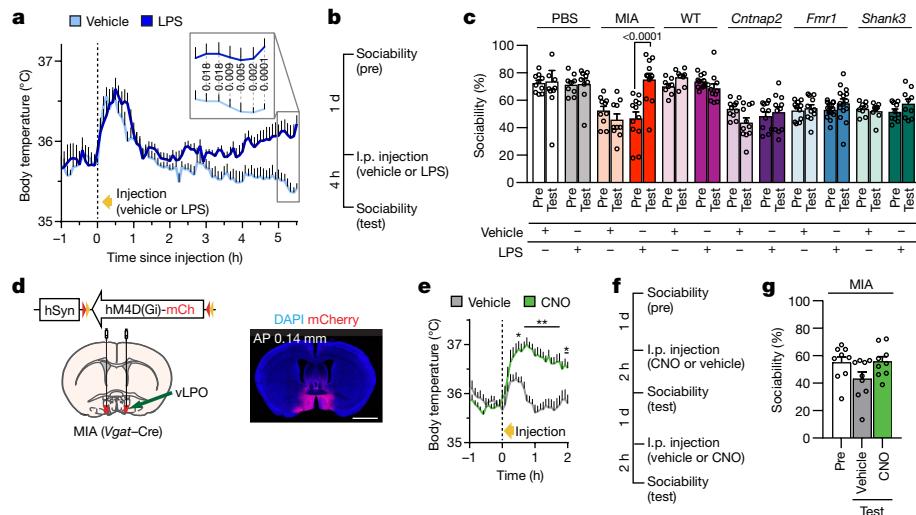
The beneficial effects of infection and the ensuing inflammation on neurological disorders have previously been noted<sup>9</sup>. For example, a subset of children with autism spectrum disorder (ASD) exhibit temporary but considerable improvements of their behavioural symptoms during episodes of fever, a sign of systemic inflammation<sup>1,2</sup>. However, a mechanistic understanding of how fever-associated immune responses translate into behavioural relief—both at the molecular and neural level—is lacking. The aetiology of ASD includes both environmental and genetic risk factors<sup>10</sup>. Mouse models for ASD, in which mice contain mutations in ASD risk genes (including *Cntnap2*<sup>5</sup>, *Fmr1*<sup>6</sup>, and *Shank3*<sup>7</sup>), show behavioural abnormalities—including deficits in

social interaction<sup>11,12</sup>. Similar behavioural abnormalities are observed in mouse models for environmental risk factors for developing ASD, such as exposure to maternal inflammation<sup>13</sup>. We sought to explore the mechanisms that enable the fever-associated rescue of sociability deficits, using both genetic and environmental mouse models for neurodevelopmental disorders.

## LPS rescues sociability in MIA offspring

A febrile response can be exogenously induced by injecting mice with a low dose of LPS<sup>14</sup>. Indeed, the intraperitoneal administration of

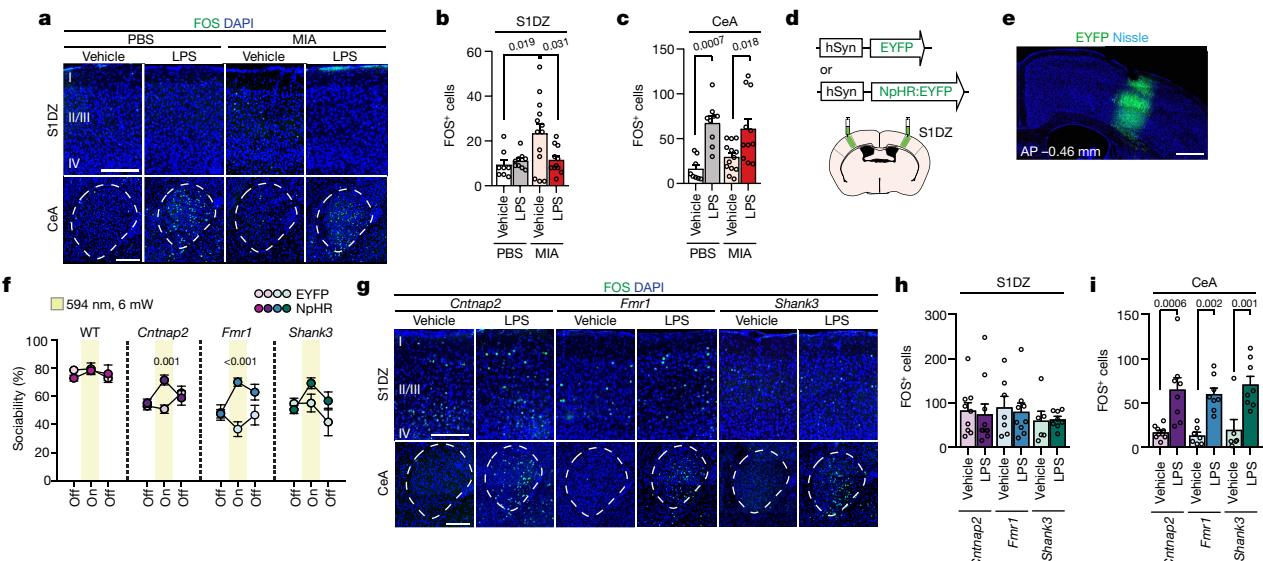
<sup>1</sup>The Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, USA. <sup>2</sup>Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA. <sup>3</sup>McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA, USA. <sup>4</sup>Department of Immunology, Blavatnik Institute, Harvard Medical School, Boston, MA, USA. <sup>5</sup>Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany. <sup>6</sup>Evergrande Center for Immunologic Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA. <sup>7</sup>These authors contributed equally: Michael Douglas Reed, Yeong Shin Yim.  
\*e-mail: Jun\_Huh@hms.harvard.edu; gbchoi@mit.edu



**Fig. 1 | Immune stimulation rescues sociability deficits in MIA offspring.**

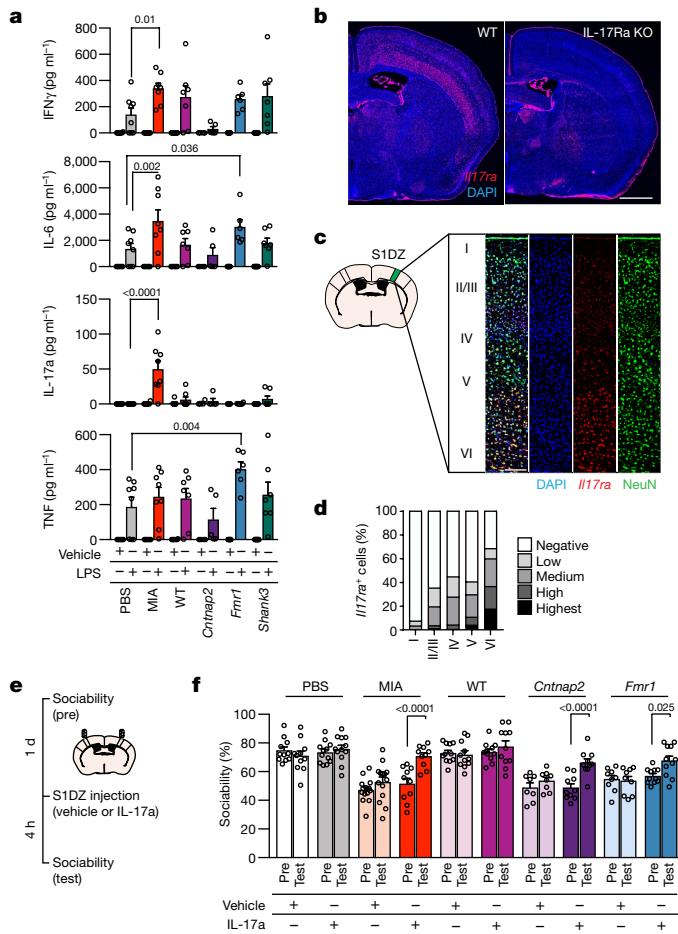
**a**, Body temperature profile after injection of saline (vehicle) or LPS in offspring of mothers injected with PBS (PBS offspring) (vehicle,  $n=11$  and LPS,  $n=11$  mice, from 5 independent experiments). The initial spike in body temperature is due to handling stress. **b, c**, Mice were tested for sociability (percentage of time spent investigating social object/total time spent investigating both social and inanimate objects) one day before LPS injection (pre-test; pre). Mice were then tested for sociability four hours after injection with vehicle or LPS (test). PBS offspring + vehicle,  $n=10$ ; PBS offspring + LPS,  $n=9$ ; MIA offspring + vehicle,  $n=10$ ; MIA offspring + LPS,  $n=12$ ; wild type (WT) + vehicle,  $n=8$ ; wild type + LPS,  $n=11$ ; *Cntrnp2* mutant + vehicle,  $n=11$ ; *Cntrnp2* mutant + LPS,  $n=11$ ; *Fmr1* mutant + vehicle,  $n=11$ ; *Fmr1* mutant + LPS,  $n=15$ ; *Shank3* mutant + vehicle,  $n=8$ ; *Shank3* mutant + LPS,  $n=10$ ; from

**3** independent experiments. **i.p.**, intraperitoneal. **d**, Virus encoding the inhibitory DREADD (AAV<sub>2</sub>-hSyn-DIO-hM4D(Gi)-mCherry) was targeted to the ventral part of the lateral preoptic nucleus (vLPO) of *Vgat*<sup>+</sup>-Cre MIA mice. Scale bar, 2 mm. **e**, Body temperature profile after injection with vehicle or CNO. **f, g**, Mice were tested for sociability one day before injection (pre). On the following two days, mice received counterbalanced injections of either vehicle or CNO. Sociability was assessed two hours after injection. For experiments in **d–g**,  $n=9$  for all groups, from 2 independent experiments. All  $n$  values refer to the number of mice used. \* $P<0.05$ , \*\* $P<0.01$ , calculated by two-way repeated-measures analysis of variance (ANOVA) with Bonferroni's (**a, e**) or Sidak's (**c**) post-hoc tests, or one-way repeated-measures ANOVA with Tukey's post-hoc test (**g**). Graphs are mean  $\pm$  s.e.m.



**Fig. 2 | Immune stimulation reduces hyperactivation in the SIDZ of MIA offspring.** **a**, Representative images illustrating FOS (green) expression in the SIDZ and CeA, after injection with vehicle or LPS. Scale bars, 200  $\mu$ m. Numerals indicate cortical layers. **b, c**, Quantification of FOS-expressing cells in the SIDZ (**b**) and CeA (**c**). For experiments in **a–c**, PBS offspring + vehicle,  $n = 8$ ; PBS offspring + LPS,  $n = 9$ ; MIA offspring + vehicle,  $n = 13$ ; MIA offspring + LPS,  $n = 11$ ; from 3 independent experiments. **d, e**, AAV encoding either EYFP or EYFP fused to enhanced halorhodopsin (NpHR) was bilaterally injected into the SIDZ of monogenic mutant mice. Scale bar, 500  $\mu$ m. **f**, Performance on sociability was assessed in the presence (on) and absence (off) of optical inhibition. For experiments in **d–f**, wild type + EYFP,  $n = 7$ ; wild type + NpHR,  $n = 8$ ; *Ctnnap2* mutant + EYFP,  $n = 11$ ; *Ctnnap2* mutant + NpHR,  $n = 9$ ; *Fmr1* mutant + EYFP,  $n = 8$ .

*Fmr1* mutant + NpHR,  $n=12$ ; *Shank3* mutant + EYFP,  $n=8$ ; *Shank3* mutant + NpHR,  $n=10$ ; from 6 independent experiments. **g**, Representative images illustrating FOS expression in the S1DZ and CeA after injection of vehicle or LPS in monogenic mutant mice. Scale bars, 200  $\mu$ m. **h, i**, Quantification of FOS-expressing cells after injection of vehicle or LPS in the S1DZ (**h**) and CeA (**i**). For experiments in **g-i**, *Cntnap2* mutant + vehicle,  $n=9$ ; *Cntnap2* mutant + LPS,  $n=10$ ; *Fmr1* mutant + vehicle,  $n=7$ ; *Fmr1* mutant + LPS,  $n=9$ ; *Shank3* mutant + vehicle,  $n=6$ ; *Shank3* mutant + LPS,  $n=8$ ; from 3 independent experiments. All  $n$  values refer to the number of mice used. Statistics calculated by two-way ANOVA with Tukey's post-hoc test (**b, c**) or Sidak's post-hoc test (**h, i**), or two-way repeated-measures ANOVA with Sidak's post-hoc test (**f**). Graphs are mean  $\pm$  s.e.m.



**Fig. 3 | IL-17a rescues sociability deficits in both MIA offspring and monogenic mutant mice.** **a**, IFN $\gamma$ , IL-6, IL-17a and TNF levels in plasma after injection of vehicle or LPS. PBS offspring + vehicle,  $n = 6$ ; PBS offspring + LPS,  $n = 8$ ; MIA offspring + vehicle,  $n = 6$ ; MIA offspring + LPS,  $n = 8$ ; wild-type + vehicle,  $n = 6$ ; wild type + LPS,  $n = 7$ ; Cntrnap2 mutant + vehicle,  $n = 4$ ; Cntrnap2 mutant + LPS,  $n = 5$ ; Fmr1 mutant + vehicle,  $n = 5$ ; Fmr1 mutant + LPS,  $n = 6$ ; Shank3 mutant + vehicle,  $n = 7$ ; Shank3 mutant + LPS,  $n = 7$ ; from 3 independent experiments. **b**, I17ra expression in wild-type and IL-17Ra-knockout (KO) mice at anterior-posterior (AP) –0.58 mm, from 1 independent experiment. Scale bar, 1 mm. **c**, Colabelling of I17ra and NeuN in the S1DZ, from 2 independent experiments. Scale bar, 100  $\mu$ m. **d**, Quantification of I17ra expression within the S1DZ according to cortical layer in PBS offspring,  $n = 8$ , from 2 independent experiments. **e**, **f**, Mice were tested for sociability one day before injection (pre) and after bilateral administration of vehicle or IL-17a into the S1DZ (test). PBS offspring + vehicle,  $n = 11$ ; PBS offspring + IL-17a,  $n = 12$ ; MIA offspring + vehicle,  $n = 14$ ; MIA offspring + IL-17a,  $n = 10$ ; wild type + vehicle,  $n = 11$ ; wild type + IL-17a,  $n = 11$ ; Cntrnap2 mutant + vehicle,  $n = 8$ ; Cntrnap2 mutant + IL-17a,  $n = 10$ ; Fmr1 mutant + vehicle,  $n = 9$ ; Fmr1 mutant + IL-17a,  $n = 11$ ; from 6 independent experiments. All  $n$  values refer to the number of mice used. Statistics calculated by two-way ANOVA with Dunnett's post-hoc test (**a**) or two-way repeated-measures ANOVA with Sidak's post-hoc test (**f**). Graphs are mean  $\pm$  s.e.m.

LPS (50  $\mu$ g kg $^{-1}$ ) to control mice—offspring born to mothers injected with PBS—led to a significant increase in body temperature (of about 0.5–1.0 °C) about 4–5 h after the treatment (Fig. 1a). To investigate whether LPS injection can ameliorate sociability deficits, we compared sociability in adult male mice one day before LPS injection (pre-test) and four hours after injection (test), at which point the increase in body temperature initiates (Fig. 1b). As previously reported<sup>4,8,15,16</sup>, MIA offspring born to mothers injected with polyinosinic:polycytidylic acid (poly(I:C)) at embryonic day (E)12.5 exhibited impaired social approach

behaviour during the pre-test (Fig. 1c, Extended Data Fig. 2). The Cntrnap2, Fmr1 and Shank3 monogenic models also displayed sociability deficits, albeit with marked variability during the pre-test (Extended Data Fig. 1). We therefore compared MIA offspring to monogenic mutant mice that exhibited a sociability index lower than about 62%, the mean value for the three monogenic lines (Extended Data Fig. 1a).

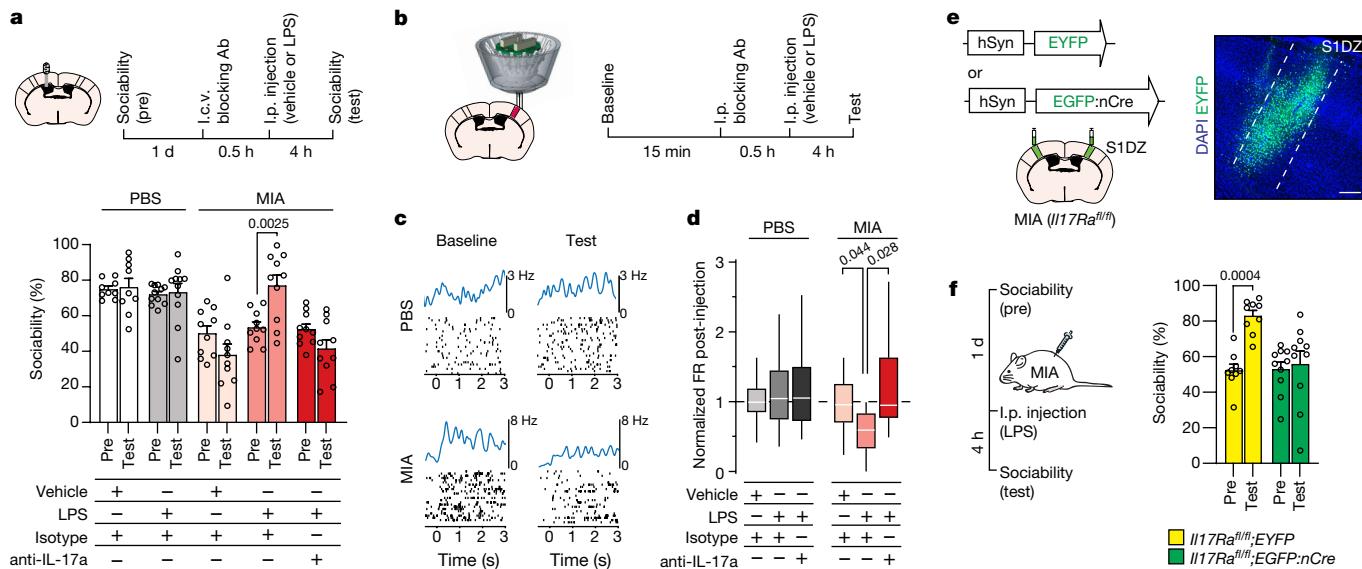
LPS injections robustly rescued the characteristic deficits in sociability of MIA offspring; treated MIA offspring displayed an interest in social objects that was indistinguishable from that of controls (Fig. 1c, Extended Data Fig. 2). LPS injection did not affect baseline sociability in control offspring. The LPS-induced rescue of sociability was absent 72 h after the treatment, paralleling the transient nature of fever-associated improvement that has previously been observed in children with ASD<sup>1</sup> (Extended Data Fig. 3a–e). Furthermore, the LPS-induced rescue was observed in both young (2–5 months old) and aged (9–12 months old) mice (Fig. 1c, Extended Data Fig. 3f–j), which suggests that this is a generalizable phenomenon that is not confined to early adulthood. The LPS-induced rescue of the sociability deficits was also evident when assayed using a reciprocal social interaction test (Extended Data Fig. 3k). Finally, the LPS treatment of MIA offspring also suppressed the enhanced marble burying behaviour (Extended Data Fig. 3l), which shows that inflammation can relieve several of the behavioural phenotypes associated with MIA. Unlike in MIA offspring however, LPS treatment did not restore sociability in mutant mice that are deficient for Cntrnap2, Fmr1 or Shank3 (Fig. 1c, Extended Data Fig. 2), indicating that rescue by LPS-driven inflammation may be applicable to only a subset of mouse models for neurodevelopmental disorders.

### Acute fever does not restore sociability

We next probed whether fever has a role in the observed behavioural rescue. Unlike in control offspring, LPS treatment did not induce changes in body temperature in MIA mice (Extended Data Fig. 4a), which suggests that the febrile response might not be the main factor that contributes to the rescue. To directly test whether fever is dispensable, we sought to increase the body temperature of the mice without inducing systemic inflammation by targeting inhibitory designer receptors exclusively activated by designer drugs (DREADDs)<sup>17</sup> to GABAergic neurons in the ventral part of the lateral preoptic nucleus of Vgat-Cre (Methods) mice<sup>18</sup> (Fig. 1d). As previously reported<sup>19</sup>, inhibition of these neurons led to an increase in body temperature of about 1 °C (Fig. 1e). However, the induction of febrile response alone did not promote social preference in MIA offspring (Fig. 1f, g, Extended Data Fig. 4b–e), confirming that fever per se is not the main driver of the LPS-induced rescue. Of note, Vgat-Cre MIA offspring exhibited sociability deficits comparable to those of wild-type MIA offspring, were unaffected by treatment with clozapine N-oxide (CNO) and showed an increase in sociability after treatment with LPS (Extended Data Fig. 4f–j).

### LPS reduces FOS induction in the S1DZ

We previously established that adult MIA offspring display cortical abnormalities that are preferentially localized in the S1DZ<sup>8</sup>, a subregion of the primary somatosensory cortex (S1) that is cyto-architecturally defined by the absence of a discernible fourth layer (Extended Data Fig. 5). The cortical phenotype is characterized by an overall increase in neural activity that, when reduced, can acutely rescue MIA-induced deficits in social behaviours<sup>8</sup>. We therefore investigated whether the LPS-induced behavioural rescue in MIA offspring is accompanied by changes in neural activity in the S1DZ. MIA offspring exhibited an increase in the number of cells in the S1DZ that express FOS (a marker for neuronal activation), relative to control offspring. However, in LPS-treated MIA offspring, the number of FOS<sup>+</sup> neurons in the S1DZ was reduced to the level of control offspring (Fig. 2a, b, Extended Data Fig. 6a–c). LPS injections did not elicit a generalized, brain-wide effect



**Fig. 4 | IL-17a is necessary for the LPS-induced rescue of sociability deficits and reduction of neural activity in the SIDZ in MIA offspring.** **a**, Mice were tested for sociability one day before injection (pre). The following day, blocking antibody against IL-17a (anti-IL-17a) or isotype-control antibody (isotype) was administered intracerebroventricularly (i.c.v.), 30 min before injection of vehicle or LPS. Sociability was assessed four hours after injection of vehicle or LPS (test). PBS offspring + vehicle + isotype,  $n=9$ ; PBS offspring + LPS + isotype,  $n=11$ ; MIA offspring + vehicle + isotype,  $n=10$ ; MIA offspring + LPS + isotype,  $n=10$ ; MIA offspring + LPS + anti-IL-17a,  $n=10$ ; from 7 independent experiments. **b–d**, Firing rate of neurons of the SIDZ before and four hours after injection of vehicle or LPS, in PBS and MIA offspring pre-treated with isotype-control antibody or blocking antibody against IL-17a. **c**, Example raster plot with firing rate profile before (baseline) and after (test) treatment with LPS, from an PBS mouse and MIA mouse pre-treated with isotype-control antibody. **d**, Normalized firing-rate (FR) change after treatment represented as box-and-whisker plots, indicating median, interquartile range and data limits

as defined by Tukey. PBS offspring + vehicle + isotype,  $n=65$  cells; PBS offspring + LPS + isotype,  $n=42$  cells; PBS offspring + LPS + anti-IL-17a,  $n=40$  cells; MIA offspring + vehicle + isotype,  $n=75$  cells; MIA offspring + LPS + isotype,  $n=48$  cells; MIA offspring + LPS + anti-IL-17a,  $n=43$  cells; from 2 PBS offspring and 2 MIA offspring in 12 independent experiments. **e**, Lentivirus encoding either EYFP or enhanced green fluorescent protein (EGFP) fused to nuclear Cre (nCre) was bilaterally injected into the SIDZ of *Il17Ra*<sup>fl/fl</sup> MIA offspring. Scale bar, 200  $\mu$ m. **f, g**, Mice were tested for sociability one day before injection (pre). The following day, mice were tested for sociability four hours after LPS injection (test). For experiments in **e, f**, *Il17ra*<sup>fl/fl</sup>,EYFP,  $n=9$  and *Il17ra*<sup>fl/fl</sup>,EGFP:nCre,  $n=10$ ; from 5 independent experiments. Unless otherwise indicated,  $n$  values refer to the number of mice used. Statistics calculated by two-way repeated-measures ANOVA with Bonferroni's post-hoc test (**a, f**) or two-way ANOVA with Tukey's post-hoc test (**d**). Graphs are mean  $\pm$  s.e.m.

on FOS expression in MIA offspring; the number of FOS<sup>+</sup> neurons either remained unchanged (as in several of the cortical regions we examined) or increased, as in the central amygdala (CeA)—a region that is known to be activated by LPS<sup>20</sup> (Fig. 2a, c, Extended Data Fig. 6d, e). Therefore, the LPS-induced behavioural rescue in MIA offspring was accompanied by a reduction in neural activity in the SIDZ.

Dysregulation of neural activity and deficits in interneuron function in the S1 have previously been associated with various genetic mouse models of neurodevelopmental disorders<sup>21–23</sup>. We therefore sought to determine whether increased neural activity can also be observed in the SIDZ of monogenic mutant mice. The number of FOS<sup>+</sup> neurons in the SIDZ of these mutant mice was increased compared to that of wild-type mice, and the magnitude of this increase correlated with the severity of the sociability deficits—notably in *Cntnap2* and *Fmr1* mutant mice (Extended Data Fig. 7a, b). These data suggest that increased neural activity in the SIDZ may also contribute to the expression of sociability deficits in monogenic mutant mice, similarly to what has been described for MIA offspring<sup>8</sup>. Consistent with this idea, optogenetically reducing neural activity in the SIDZ could rescue sociability deficits in *Cntnap2* and *Fmr1* mutant mice (Fig. 2d–f, Extended Data Fig. 7c–f). *Shank3* mutant mice also showed an increase in sociability upon photoinhibition, but this increase was not significantly different from that of *Shank3* control mice that express enhanced yellow fluorescent protein (EYFP). Therefore, reducing neural activity in the SIDZ was sufficient to restore sociability in *Cntnap2* and *Fmr1* mutant mice, as well as in MIA offspring<sup>8</sup>. Unlike in MIA offspring however, LPS treatment did not reduce the number of FOS<sup>+</sup> neurons in the SIDZ of monogenic mutant mice (Fig. 2g–i).

## IL-17a rescues sociability deficits

The injection of LPS is known to increase the production of inflammatory cytokines<sup>24</sup>. We observed that administration of LPS results in a robust increase in the plasma levels of IFN $\gamma$ , IL-6 and TNF (also known as TNF- $\alpha$ ) (Fig. 3a). IL-17a—the orthologue of which in *Caenorhabditis elegans* has previously been implicated in modulating sensory responses<sup>25</sup>—was prominently upregulated in MIA offspring, but not in monogenic mutant mice or in control mice (Fig. 3a). Furthermore, we noted that the receptor subunit A for IL-17a (IL-17RA) is expressed in cortical neurons, including in the SIDZ (Fig. 3b–d, Extended Data Fig. 8a–c). These data suggested that the increased levels of IL-17a after treatment with LPS in MIA offspring, but not in monogenic mutant mice, may directly affect the SIDZ and thus restore sociability. Consistent with this idea, the direct administration of recombinant IL-17a into the SIDZ was sufficient to increase sociability not only in MIA offspring but also in *Cntnap2* and *Fmr1* mutant mice (Fig. 3e, f, Extended Data Fig. 8d–h).

## LPS-induced rescue requires IL-17a

To further determine whether IL-17a mediates the LPS-driven behavioural rescue, we inhibited IL-17a activity in the brain via intracerebroventricular injection of blocking antibodies. Antibodies against IL-17a prevented both the LPS-induced rescue of sociability (Fig. 4a, Extended Data Fig. 9a–e) and the reduction of FOS expression in the SIDZ of MIA offspring (Extended Data Fig. 9f). To directly assay the effects of LPS on neural activity, we used multi-electrode arrays to measure the firing rate of neurons of the SIDZ in awake mice

(Extended Data Fig. 10a, b). Upon treatment with LPS, we observed a decrease in the overall firing rate that was prevented by blocking IL-17a in MIA offspring. Treatment with LPS or IL-17a blocking antibody did not change the neuronal firing rate in the S1DZ of control offspring (Fig. 4b–d, Extended Data Fig. 10c). Furthermore, LPS injection did not restore sociability to MIA offspring that are deficient for IL-17Ra in the neurons of the S1DZ (Fig. 4e, f, Extended Data Fig. 10d–j). Our data collectively demonstrate that IL-17a mediates the restoring effects of inflammation on social behaviours by directly acting on IL-17Ra<sup>+</sup> neurons of the S1DZ.

## Discussion

Previous data in mice have suggested that increased production of IL-17a in pregnant mothers may present as a risk factor for neurodevelopmental disorders in offspring<sup>15,26,27</sup>. On the basis of our current findings, we propose that in adult MIA offspring the same cytokine is beneficial and ameliorates sociability phenotypes during episodes of inflammation. Treatment with LPS led to an increase in IL-17a levels in the blood selectively in MIA offspring—but not in other monogenic mutant mice. This suggests that the inflammatory responses may result in beneficial effects only for individuals who have their immune systems primed by prenatal exposure to immune activation, or by other environmental factors. A better understanding of the role of a primed immune system among patients with neurodevelopmental disorders may help to identify those patients whose behavioural symptoms are likely to improve after exposure to fever-associated inflammation. Furthermore, elucidating the mechanisms by which IL-17a can induce two opposing behavioural outcomes depending on when its upregulation occurs (during embryonic brain development or in the adult brain) may provide opportunities to devise therapeutic as well as preventive treatments for the behavioural symptoms associated with ASD.

## Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-019-1843-6>.

- Curran, L. K. et al. Behaviors associated with fever in children with autism spectrum disorders. *Pediatrics* **120**, e1386–e1392 (2007).
- Grzadzinski, R., Lord, C., Sanders, S. J., Werling, D. & Bal, V. H. Children with autism spectrum disorder who improve with fever: insights from the Simons Simplex Collection. *Autism Res.* **11**, 175–184 (2018).

- Shi, L., Fatemi, S. H., Sidwell, R. W. & Patterson, P. H. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. *J. Neurosci.* **23**, 297–302 (2003).
- Smith, S. E. P., Li, J., Garbett, K., Mirnics, K. & Patterson, P. H. Maternal immune activation alters fetal brain development through interleukin-6. *J. Neurosci.* **27**, 10695–10702 (2007).
- Peñagarikano, O. et al. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. *Cell* **147**, 235–246 (2011).
- The Dutch-Belgian Fragile X Consortium. *Fmr1 knockout mice: a model to study fragile X mental retardation*. *Cell* **78**, 23–33 (1994).
- Peça, J. et al. *Shank3* mutant mice display autistic-like behaviours and striatal dysfunction. *Nature* **472**, 437–442 (2011).
- Yim, Y. et al. Reversing behavioural abnormalities in mice exposed to maternal inflammation. *Nature* **549**, 482–487 (2017).
- Whitrow, M. Wagner-Jauregg and fever therapy. *Med. Hist.* **34**, 294–310 (1990).
- Lyall, K. et al. The changing epidemiology of autism spectrum disorders. *Annu. Rev. Public Health* **38**, 81–102 (2017).
- Crawley, J. N. Translational animal models of autism and neurodevelopmental disorders. *Dialogues Clin. Neurosci.* **14**, 293–305 (2012).
- Ey, E., Leblond, C. S. & Bourgeron, T. Behavioral profiles of mouse models for autism spectrum disorders. *Autism Res.* **4**, 5–16 (2011).
- Patterson, P. H. Maternal infection and immune involvement in autism. *Trends Mol. Med.* **17**, 389–394 (2011).
- Kozak, W., Conn, C. A. & Kluger, M. J. Lipopolysaccharide induces fever and depresses locomotor activity in unrestrained mice. *Am. J. Physiol.* **266**, R125–R135 (1994).
- Choi, G. B. et al. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science* **351**, 933–939 (2016).
- Malkova, N. V., Yu, C. Z., Hsiao, E. Y., Moore, M. J. & Patterson, P. H. Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. *Brain Behav. Immun.* **26**, 607–616 (2012).
- Armbuster, B. N., Li, X., Pausch, M. H., Herlitze, S. & Roth, B. L. Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. *Proc. Natl. Acad. Sci. USA* **104**, 5163–5168 (2007).
- Vong, L. et al. Leptin action on GABAergic neurons prevents obesity and reduces inhibitory tone to POMC neurons. *Neuron* **71**, 142–154 (2011).
- Zhao, Z. D. et al. A hypothalamic circuit that controls body temperature. *Proc. Natl. Acad. Sci. USA* **114**, 2042–2047 (2017).
- Cai, H., Haubensak, W., Anthony, T. E. & Anderson, D. J. Central amygdala PKC-δ<sup>+</sup> neurons mediate the influence of multiple anorexigenic signals. *Nat. Neurosci.* **17**, 1240–1248 (2014).
- Gogolla, N. et al. Common circuit defect of excitatory-inhibitory balance in mouse models of autism. *J. Neurodev. Disord.* **1**, 172–181 (2009).
- Selby, L., Zhang, C. & Sun, Q. Major defects in neocortical GABAergic inhibitory circuits in mice lacking the fragile X mental retardation protein. *Neurosci. Lett.* **412**, 227–232 (2007).
- Orefice, L. L. et al. Targeting peripheral somatosensory neurons to improve tactile-related phenotypes in ASD models. *Cell* **178**, 867–886 (2019).
- Erickson, M. A. & Banks, W. A. Cytokine and chemokine responses in serum and brain after single and repeated injections of lipopolysaccharide: multiplex quantification with path analysis. *Brain Behav. Immun.* **25**, 1637–1648 (2011).
- Chen, C. et al. IL-17 is a neuromodulator of *Caenorhabditis elegans* sensory responses. *Nature* **542**, 43–48 (2017).
- Kim, S. et al. Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. *Nature* **549**, 528–532 (2017).
- Lammert, C. R. et al. Cutting edge: critical roles for microbiota-mediated regulation of the immune system in a prenatal immune activation model of autism. *J. Immunol.* **201**, 845–850 (2018).

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2019

# Article

## Methods

### Mice

All experiments were performed according to the Guide for the Care and Use of Laboratory Animals and were approved by the National Institutes of Health and the Committee on Animal Care at Massachusetts Institute of Technology. C57BL/6 mice were purchased from Taconic Biosciences for generating PBS and MIA offspring. *Slc32a1(Vgat)*-Cre (028862) mice were purchased from Jackson Laboratories, and inbred. For monogenic experiments, C57BL/6, *Cnnap2* (017482), *Fmr1* (003025) and *Shank3* (017688) mice were purchased from Jackson Laboratories, and inbred after colonizing with segmented filamentous bacteria (SFB) from donor mice. *Il-17ra*<sup>f/f</sup> and IL-17Ra-knockout mice have previously been described<sup>28,29</sup>. All mice were males aged 2–5 months, unless otherwise specified.

### Maternal immune activation

Mice were mated overnight with females carrying SFB in their guts<sup>26</sup>. On E12.5, pregnant female mice were weighed and injected with a single dose (20 mg/kg, intraperitoneal injection) of poly(I:C) (P9582, Sigma Aldrich) or PBS. Each dam was returned to its cage and left undisturbed until the birth of its litter. All pups remained with the mother until weaning on postnatal day (P)21–28, at which time mice were group-housed at a maximum of 5 per cage with same-sex littermates. Matings between *Vgat*-Cre (c/c) males and wild-type females were used to make MIA *Vgat*-Cre mice.

### Stereotaxic surgery

Surgeries were carried out using aseptic techniques. Mice were anaesthetized using a mixture of ketamine (100 mg/kg, intraperitoneal injection) and xylazine (10 mg/kg, intraperitoneal injection). Mice were given pre-operative slow-release buprenorphine (1.0 mg/kg, subcutaneous injection). For manipulating body temperature with vLPO inhibition, *Vgat*-Cre mice received bilateral stereotaxic injections of virus (200 nl per side) at rates of <0.1 ml/min (AAV<sub>2</sub>-hSyn-DIO-hM4Di-mCherry (Addgene)). Virus was bilaterally targeted to the vLPO (AP +0.15, medial-lateral (ML) ±0.50, dorsal–ventral (DV), -4.9, lambda was raised 600 µm above bregma). For experiments involving optical manipulation in the S1DZ, wild-type, *Cnnap2*, *Fmr1* and *Shank3* mice received bilateral stereotaxic injections of virus (400 µl/side) at a rate of <0.1 ml/min (AAV<sub>2</sub>-hSyn-EYFP, AAV<sub>2</sub>-hSyn-eNpHR3.0:EYFP). Viruses were bilaterally targeted to the S1DZ (AP -0.50, ML ±2.6, DV -0.80, lambda was level with bregma). To deliver light into the S1DZ, a 300-µm optic fibre was superficially implanted in the S1DZ. For experiments involving genetic knockout of IL-17Ra in the S1DZ, *Il-17ra*<sup>f/f</sup> mice received bilateral stereotaxic injections of virus (800 nl per side) at a rate of <0.1 ml/min (pLenti-hSyn-EYFP, pLenti-hSyn-EGFP:nCre). Viruses were bilaterally targeted to the S1DZ. All lentiviruses were made in-house. For administration of cytokines into the S1DZ, cannulas (PlasticsOne) were implanted superficially within the S1DZ. For central administration of blocking antibodies, cannulas were implanted above the right lateral ventricle (AP -0.30, ML +1.0, DV -1.35, lambda was raised 600 µm above bregma). Cannulas were fitted with dummy cannulas (PlasticsOne) to maintain cannula patency after surgery.

### Tracking of body temperature

Body temperature was measured using the Anipill remote temperature monitoring system (007894-001, DSI). Adult male mice were implanted with an Anipill capsule in the abdominal cavity. Experiments were carried out more than three weeks after surgery. Mice were singly housed the day before the experiment. Ambient temperature was maintained at 23.5 °C, consistent with the ambient temperature of the vivarium. Body temperature was sampled in 5-min increments. LPS or vehicle

injections occurred between 11:00–13:00 for experiments assaying the effects of LPS on body temperature in PBS and MIA offspring.

### Immunohistochemistry

Mice were transcardially perfused with cold paraformaldehyde (PFA) (4% in PBS). Brains were kept in PFA overnight at 4 °C before vibratome-sectioning (Leica VT1000s). Brains were cut at 50-µm thickness for FOS quantification. Brains were cut at 100-µm thickness for all other experiments.

Before antibody labelling, sections were incubated in blocking solution (0.4% Triton X-100 and 2% goat serum in PBS) for 30 min. Sections were then incubated in blocking solution containing primary antibodies overnight at room temperature. Primary antibodies used were chicken anti-GFP (1:1,000, Ab5450, Abcam), rabbit anti-FOS (1:500, ABE457, Millipore), rabbit anti-DsRed (1:1,000, 632496, Clontech) and mouse anti-NeuN (1:1,000, MAB377, Millipore). Sections were washed in wash buffer (0.4% Triton X-100 in PBS) three times before secondary antibody labelling. Sections were incubated in blocking solution containing secondary antibodies and DAPI (1:5,000, D1306, Thermo Fisher) for three hours at room temperature. Images of stained slices were acquired using a confocal microscope (LSM710, Carl Zeiss) with a 10×, 20× or 40× objective lens.

### In situ hybridization

Mice were transcardially perfused with cold PBS. Brains were extracted and embedded in optimal cutting temperature (OCT) compound on dry ice. Sections were cut at 20-µm thickness on a cryostat. In situ hybridizations were performed using the RNAscope 2.5 HD Assay-Red kit (322350, Advanced Cell Diagnostics) using a probe targeting the *Il17ra* transcript (Mm-Il17ra-O1, 566131, Advanced Cell Diagnostics). The probe was designed to target region 444–882 of the *Il17ra* transcript (NM\_008359.2). Modifications to the kit protocol to improve adherence of tissue to the slide included an extension of the fixation time to 30 min and the addition of a humidified bake step at 40 °C immediately before probe hybridization. Sections were counterstained with DAPI. Images were acquired using a confocal microscope (LSM710, Carl Zeiss) with a 10× or 20× objective lens. *Il17ra* and DAPI expression was quantified using QuPath<sup>30</sup>. Cells were divided into the following categories based on level of *Il17ra* expression: low = 1–3 puncta, medium = 4–9 puncta, high = 10–15 puncta, highest = >15 puncta.

### In situ hybridization followed by immunohistochemistry

For experiments assaying the overlap of *Il17ra* and NeuN expression, immunohistochemistry for NeuN was performed following a modified in situ hybridization protocol. The RNAscope 2.5 HD Assay-Red kit in situ protocol was modified in the following ways: sections were baked at 60 °C, followed by a 10-min fixation step with 4% PFA at room temperature. Sections were then stored in 70% ethanol at 4 °C overnight. Sections were permeabilized in 8% SDS for 10 min. Sections were washed twice with PBS between each step. After SDS treatment, the RNAscope 2.5 HD Assay-Red kit protocol was followed from the probe hybridization step. Following the completion of the in situ protocol, sections were incubated in blocking buffer containing anti-NeuN antibody overnight at 4 °C. Sections were then incubated in blocking buffer containing DAPI and secondary antibody for two hours at room temperature.

### Behavioural analysis

Male mice were tested during the light cycle in a room with lighting maintained at 230 lux. Mice were transferred to the testing area at least one hour before the initiation of experiments. Tracking of mouse behaviour was done using the EthoVision XT (Noldus) tracking system.

**Three-chamber social approach assay.** Adult male mice were assayed for sociability using a three-chamber social approach assay. The arena

was constructed of white acrylic (50 cm × 35 cm × 30 cm). Wire cups (Spectrum Diversified) were placed in the back left and right corner of the arena beneath water-filled 1-l bottles (Nalgene). On day 0, mice were habituated to the arena for 10 min. Immediately after habituation, mice were singly housed. On day 1 (pre-test), mice were placed in the centre of the arena and allowed to freely explore. Following 10 min, mice were confined to the centre of the arena. An inanimate object (rubber stopper) or a male conspecific were placed beneath the wire cups. Placement of the inanimate object and social target were alternated. Mice were then allowed to freely explore the arena for 10 min. Interaction time was defined as time spent in the areas circumscribing the wire cups (<2 cm). Sociability was defined as interaction time with the social target divided by total interaction time and expressed as a percentage. For experiments involving LPS injections, mice were injected with either saline (vehicle) or LPS (50 µg/kg, intraperitoneal injection, L2630, Sigma) on day 2 (test), four hours before testing. For the experiment assaying sociability 72 h after injection of LPS, mice used for 4-h LPS sociability experiments were tested for sociability again at 72 h.

**Three-chamber social approach assay with DREADD manipulation.** Adult male *Vgat*-Cre MIA offspring were bilaterally injected with virus encoding the inhibitory DREADD receptor fused to mCherry into the vLPO. After more than three weeks of recovery, mice were assayed on the three-chamber social approach assay outlined above. Baseline sociability was assayed on day 1 (pre-test). On day 2 and day 3, mice were injected with either vehicle or CNO (1.5 mg/kg, intraperitoneal injection, BML-NS105, Enzo Life Sciences), two hours before initiation of behaviour. Injection order was counterbalanced. Following behavioural experiments, post-mortem histology was used to confirm mCherry expression within the vLPO. For experiments assaying the effect of injection of CNO and LPS in *Vgat*-Cre PBS and MIA offspring that have not undergone surgery, baseline sociability was assessed on day 1. On day 2 and 3, mice received counterbalanced injections of CNO or vehicle. On day 4, mice were injected with LPS.

**Three-chamber social approach assay with administration of IL-17a into the S1DZ.** Adult male mice were implanted with a cannula into S1DZ bilaterally and allowed to recover for more than two weeks before the behavioural experiments. On day 1 (pre-test), mice were assayed for sociability. On day 2 (test), mice were anaesthetized briefly using isoflurane and either vehicle or IL-17a (50 ng per side in 1 µl at a rate of 180 nl/min, 7956-ML/CF, R&D) was administered bilaterally into the S1DZ through 250-µm projecting injector tips (PlasticsOne). Four hours after administration of vehicle or IL-17a, mice were assayed for sociability. Cannula placements were verified using histology.

**Three-chamber social approach assay with S1DZ optogenetic inhibition.** EYFP or NpHR were virally targeted to the S1DZ. After two weeks of recovery, mice were allowed to freely explore the arena for 10 min. The following day, the mice were given 3 min of no stimulation ('off' session) and 3 min of laser stimulation ('on' session) (594 nm, 6 mW).

**Three-chamber social approach assay with IL-17a blocking antibody.** For central cytokine blockade experiments, adult male mice were implanted with a cannula into the lateral ventricle and allowed to recover for more than two weeks before the behavioural experiments. On day 1 (pre-test), mice were tested for baseline sociability. On day 2 (test), mice were injected with IL-17a blocking antibody (clone 50104; R&D) or isotype-control antibody (IgG2a, clone 54447; R&D). Antibodies were dissolved in saline and administered intracerebroventricularly at 1 mg/kg in 500 nl at a rate of 180 nl/min through 750-µm projecting injector tips (PlasticsOne). Blocking antibody was administered 30 min before administration of LPS. Four hours following administration of LPS, mice were assayed for sociability.

**Three-chamber social approach assay in mice deficient for IL-17Ra in the S1DZ.** IL-17Ra-knockout was mediated by viral delivery of Cre recombinase, expressed under the control of the human synapsin (hSyn) promoter, into the S1DZ of *Il-17ra*<sup>fl/fl</sup> MIA offspring. After at least three weeks of recovery, mice were assayed for sociability. On the next day sociability was assessed four hours following administration of LPS.

**Marble burying assay.** On day 1, mice were tested for their baseline marble burying phenotype. On day 2, four hours before beginning the marble burying assay, mice were treated with either LPS or vehicle. The marble burying assay was carried out as previously described<sup>8</sup>. Mice were placed into testing arenas (arena size 40 cm × 20 cm × 30 cm; bedding depth 3 cm) each containing 20 glass marbles (laid out in 4 rows of 5 marbles equidistant from one another). At the end of the 15-min exploration period mice were carefully removed from the testing cages and the number of marbles buried was recorded. The marble burying index was arbitrarily defined as the following: 1 for marbles covered >50% with bedding, 0.5 for marbles covered <50% with bedding or 0 for anything less.

**Reciprocal social interaction assay.** Four hours before testing, mice were injected with vehicle or LPS. Two unfamiliar mice of the same treatment and background were placed in a fresh mouse cage and allowed to freely interact for 10 min. Videos were acquired using IC Capture (The Imaging Source) at a 640 × 480 aspect ratio and 25 frames per second. Social interaction (close following, push–crawl, nose–nose sniffing and nose–anus sniffing) was scored by an observer blind to treatment and background<sup>31</sup>.

#### Quantification of FOS<sup>+</sup> cells in the brain after administration of LPS

Adult male mice were killed five hours after injection of LPS. FOS<sup>+</sup> cells were quantified using the Cell Counter plugin in Fiji<sup>32</sup>. All cells were counted within a single coronal section of each respective brain region, as defined by the Paxinos and Franklin Mouse Atlas<sup>33</sup>. Regions quantified include: medial prefrontal cortex (mPFC) (comprising prelimbic cortex (PrL) and infralimbic cortex (IL)), AP +1.98; S1DZ, AP –0.46; primary somatosensory cortex barrel field (S1BF), AP –0.46; primary motor cortex (M1), AP –0.46; secondary motor cortex (M2), AP –0.46; secondary auditory cortex dorsal part (AuD), AP –1.94; CeA, AP –1.94; and primary visual cortex (V1), AP –3.64. For experiments testing the IL-17a dependence of LPS-induced changes in FOS expression, mice were injected intracerebroventricularly with control antibodies or blocking antibody against IL-17a 30 min before intraperitoneal injection of vehicle or LPS. Surgical and injection methods were identical to behavioural experiments.

#### Enzyme-linked immunosorbent assay

After four hours of administration of vehicle or LPS, mice were anaesthetized by intraperitoneal injection of Fatal-Plus (100 mg/kg). All blood samples were centrifuged at 10,000g for 10 min at 4 °C. All samples were stored at –80 °C until further analysis. Cytokine concentrations in plasma were measured using an ELISA kit (IFNy; 430804, TNF; 430904, IL-6; 431304 and IL-17a; 432504, Biolegend), following the manufacturer's instructions.

#### PCR for assaying IL-17Ra knockout

*Il17ra*<sup>fl/fl</sup> male mice were bilaterally injected with virus encoding nuclear Cre fused to EGFP, or control virus encoding only EYFP, into the S1DZ. After more than three weeks, injection sites were dissected from the S1. Single cells were dissociated from brain tissue using a modified version<sup>34</sup> of the Papain Dissociation Kit protocol (LK003153, Worthington) and sorted on a BD FACS Aria (BD Biosciences) based on EGFP and EYFP expression. RNA was extracted from sorted cells using

# Article

a Quick-RNA micro-prep kit (Zymo). Twenty nanograms of RNA was converted into cDNA using oligodT (Protoscript First Strand CDNA Synthesis Kit, NEB). *Il17ra* mRNA expression was analysed using PCR. One microlitre of cDNA was diluted in a 20- $\mu$ l reaction volume. *Il17ra* and *Gapdh* mRNA expression was assessed using the following primers: *Il17ra* 5'-AGATGCCAGCATCCTGTACC-3' and 5'-CACAGTCACAGCGTGTCTCA-3'; *Gapdh* 5'-GACTTCAACAGCCTCCACTCTTCC-3' and 5'-TGGGTGGTCCAGGTTTACTCCTT-3'. Cycling conditions for *Il17ra*: 95 °C  $\times$  5 min (1 cycle), 95 °C  $\times$  20 s, 60 °C  $\times$  30 s, 72 °C  $\times$  30 s (32 cycles), 72 °C  $\times$  5 min (1 cycle) and 4 °C hold. Cycling conditions for *Gapdh*: 95 °C  $\times$  5 min (1 cycle), 95 °C  $\times$  20 s, 60 °C  $\times$  30 s, 72 °C  $\times$  30 s (28 cycles), 72 °C  $\times$  5 min (1 cycle) and 4 °C hold. Band intensity from gel images was quantified using ImageJ.

## In vivo electrophysiology

Electrophysiological experiments were conducted in head-fixed mice trained to walk on a rotating running wheel. Before training, mice were implanted with custom crowns permitting head-fixing above the wheel and allowed to recover for one week before training. Mice were trained to walk on the wheel for three 10-min sessions daily for at least one week. Following training, mice were implanted with a multi-electrode array targeting the S1DZ and allowed to recover before testing. To assess the IL-17a dependence of LPS-induced changes in neural activity, mice were injected intraperitoneally with control antibodies or blocking antibody against IL-17a (1 mg per mouse), 30 min before intraperitoneal injection of vehicle or LPS. Baseline neural activity was measured while mice were running on the wheel immediately before the first injection. After the first injection, mice were returned to their home cage. Post-injection (test) neural activity during wheel running was measured 4 h after injection of vehicle or LPS.

**Multi-electrode array construction and implantation.** Custom multi-electrode array scaffolds (drive bodies) were designed using 3D CAD software (SolidWorks) and printed in Accura 55 plastic (American Precision Prototyping), as previously described<sup>35,36</sup>. Before implantation, each array scaffold was loaded with 8–24 independently movable micro-drives carrying 12.5- $\mu$ m nichrome (California Fine Wire Company) tetrodes. Electrodes were pinned to custom-designed 32- or 128-channel electrode interface boards (Sunstone Circuits) along with a common reference wire (A-M systems).

**Electrophysiological recordings and spike sorting.** Signals were acquired using a Neuralynx multiplexing digital recording system (Neuralynx) through a combination of 32- and 64-channel digital multiplexing headstages plugged into the electrode interface board of the implant. Signals from each electrode were amplified, filtered between 0.1 Hz and 9 kHz and digitized at 30 kHz. Initial spike sorting was performed using MountainSort, followed by manual quality control using MClust toolbox (<http://redishlab.neuroscience.umn.edu/mclust/MClust.html>).

**Analysis of firing rate.** At time 0 in each recording session, mice began walking on the wheel rotating at 7.5 cm s<sup>-1</sup>. Data collected between 1 and 2 s were included in the analysis. The firing rate was calculated across 1-s time windows during which mice were walking stably on the rotating wheel, averaged across 25–50 trials per condition. The first second after the onset of wheel rotation was omitted, to avoid firing rate changes due to acceleration. Firing rate was sampled with a 1-ms bin width passed through a box car filter (100 ms). The resulting peri-stimulus time histograms were then smoothed with a 50-ms Gaussian.

To assess the effect of treatment on neural activity, changes in firing rates during wheel running 4 h after injection were normalized to the pre-injection baseline. Cells showing a firing rate below 0.1 Hz were excluded from analysis.

## Statistics and reproducibility

Statistical analyses were performed using GraphPad Prism. Sample size was chosen on the basis of similar previous studies, and not on statistical methods to predetermine sample size<sup>8,15</sup>. Within each iteration of an experiment, mice were randomly assigned to groups with approximately balanced sample size. Behavioural results from mice with inaccurate targeting of viral infection or cannula implantations were excluded. Experimenters were blind to subject treatment during data collection and analysis.

## Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

## Data availability

Source Data for Figs. 1–4 and Extended Data Figs. 1–4, 6–10, containing raw data for all experiments, are provided with the paper. All other data are available from the corresponding author on reasonable request.

28. El Malki, K. et al. An alternative pathway of imiquimod-induced psoriasis-like skin inflammation in the absence of interleukin-17 receptor a signaling. *J. Invest. Dermatol.* **133**, 441–451 (2013).
29. Tusi, B. K. et al. Population snapshots predict early haematopoietic and erythroid hierarchies. *Nature* **555**, 54–60 (2018).
30. Bankhead, P. et al. QuPath: open source software for digital pathology image analysis. *Sci. Rep.* **7**, 16878 (2017).
31. Silverman, J. L., Yang, M., Lord, C. & Crawley, J. N. Behavioural phenotyping assays for mouse models of autism. *Nat. Rev. Neurosci.* **11**, 490–502 (2010).
32. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**, 676–682 (2012).
33. Paxinos, G. & Franklin, K. B. J. *The Mouse Brain in Stereotaxic Coordinates* (Academic, 2001).
34. Hrvatin, S. et al. Single-cell analysis of experience-dependent transcriptomic states in the mouse visual cortex. *Nat. Neurosci.* **21**, 120–129 (2018).
35. Halassa, M. M. et al. State-dependent architecture of thalamic reticular subnetworks. *Cell* **158**, 808–821 (2014).
36. Brunetti, P. M. et al. Design and fabrication of ultralight weight, adjustable multi-electrode probes for electrophysiological recordings in mice. *J. Vis. Exp.* **91**, e51675 (2014).

**Acknowledgements** We thank N. Soares, M. Garcia and Y. Liu for assistance with experiments, and B. Noro and M. Trombly for critical reading of the manuscript. This work was supported by the National Institute of Mental Health (1-R01-MH115037-01, to G.B.C.), Jeongho Kim Neurodevelopmental Research Fund (G.B.C. and J.R.H.), Hock E. Tan and K. Lisa Yang Center for Autism Research (G.B.C. and M.D.R.), P. Ha (G.B.C.), Simons Center for the Social Brain (G.B.C., J.R.H. and Y.S.Y.), the Simons Foundation Autism Research Initiative (G.B.C. and J.R.H.), the Champions of the Brain Weedon Fellowship (G.M.W.) and the National Science Foundation Graduate Research Fellowship (no. 1122374, to M.D.R.). We are deeply grateful to B. Picover, the JPB Foundation, the Picover Institute for Learning and Memory, Lore McGovern and the McGovern Institute for Brain Research for their mentorship and direct support of this work over the years.

**Author contributions** M.D.R., Y.S.Y., M.M.H., J.R.H. and G.B.C. designed the experiments and/or provided advice and technical expertise. M.D.R., Y.S.Y., R.D.W., H.K., C.R., G.M.W., M.A. and H.O.K. performed the experiments. A.W. provided *Il17ra*<sup>fl/fl</sup> mice. M.D.R., Y.S.Y., J.R.H. and G.B.C. wrote the manuscript with input from the co-authors.

**Competing interests** The authors declare no competing interests.

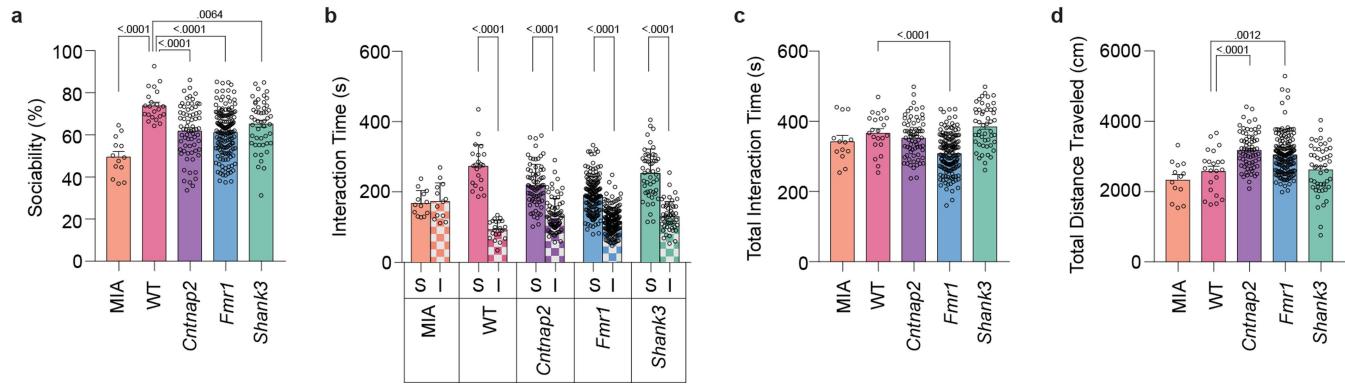
## Additional information

**Supplementary information** is available for this paper at <https://doi.org/10.1038/s41586-019-1843-6>.

**Correspondence and requests for materials** should be addressed to J.R.H. or G.B.C.

**Peer review information** *Nature* thanks Thomas Blank, Craig M. Powell, Marco Prinz and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

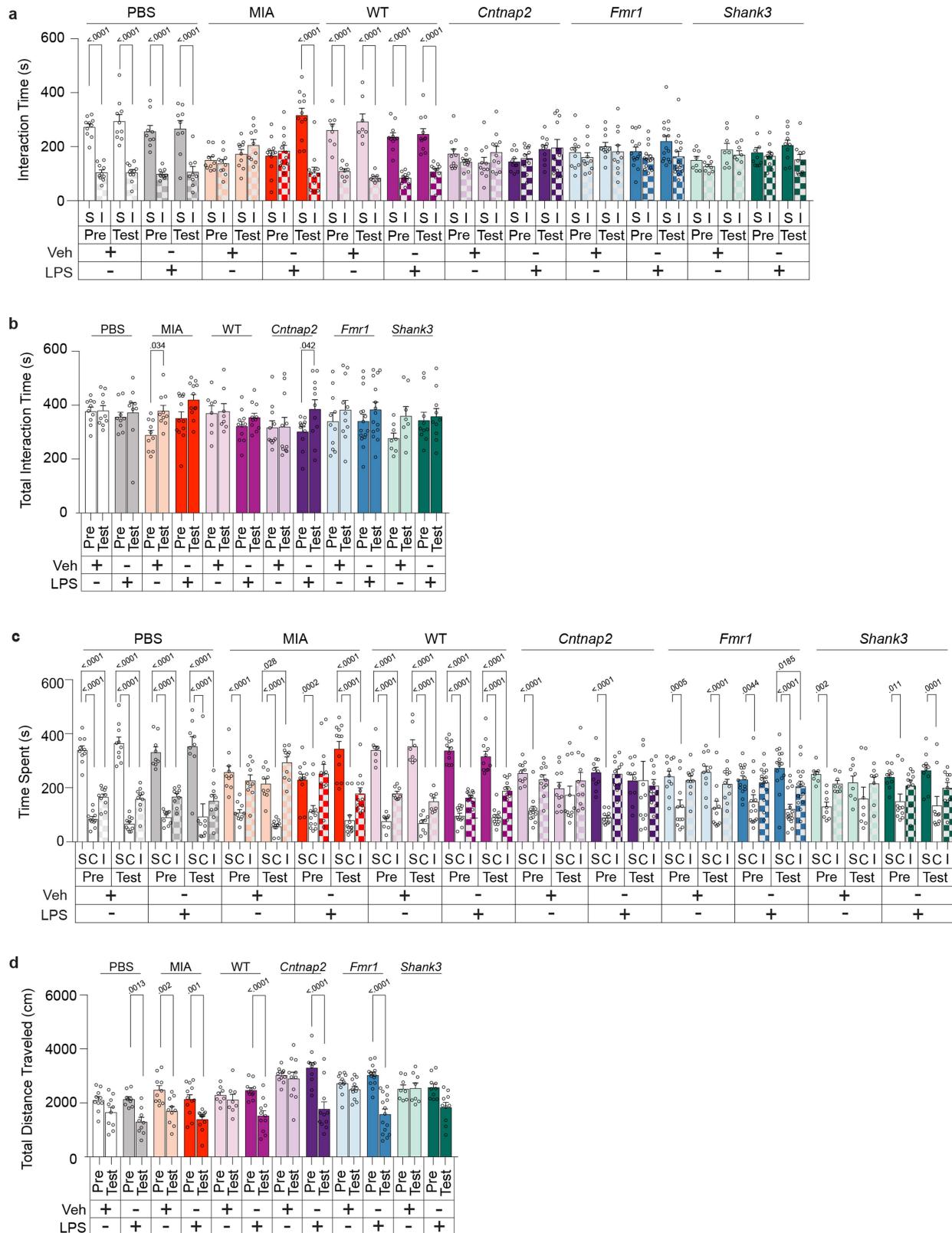
**Reprints and permissions information** is available at <http://www.nature.com/reprints>.



**Extended Data Fig. 1 | *Cntnap2*, *Fmr1* and *Shank3* mutant mice show variable sociability performance.** **a**, Sociability performance. MIA,  $n=13$ ; wild-type,  $n=22$ ; *Cntnap2* mutant,  $n=71$ ; *Fmr1* mutant,  $n=165$ ; *Shank3* mutant,  $n=50$ ; from 30 independent experiments. **b**, Time spent investigating social (S) versus inanimate (I) objects for mice described in **a**. **c**, Total interaction

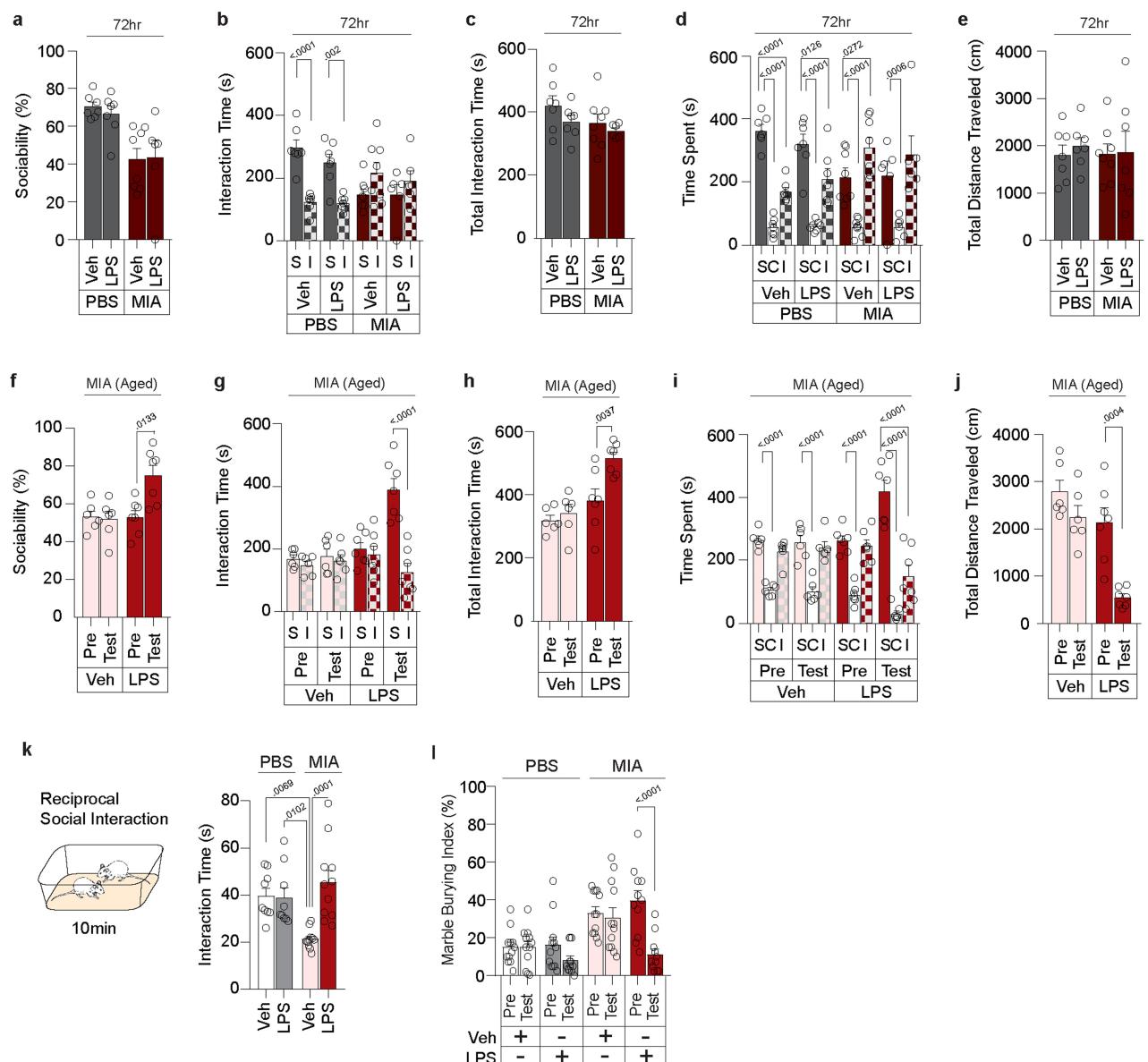
time (**c**) and distance travelled (**d**) during the three-chambered sociability experiments described in **a**. All  $n$  values refer to the number of mice used. Statistics calculated by one-way ANOVA with Dunnett's post-hoc test (**a, c, d**) or two-way ANOVA with Dunnett's post-hoc test (**b**). Graphs are mean  $\pm$  s.e.m.

# Article



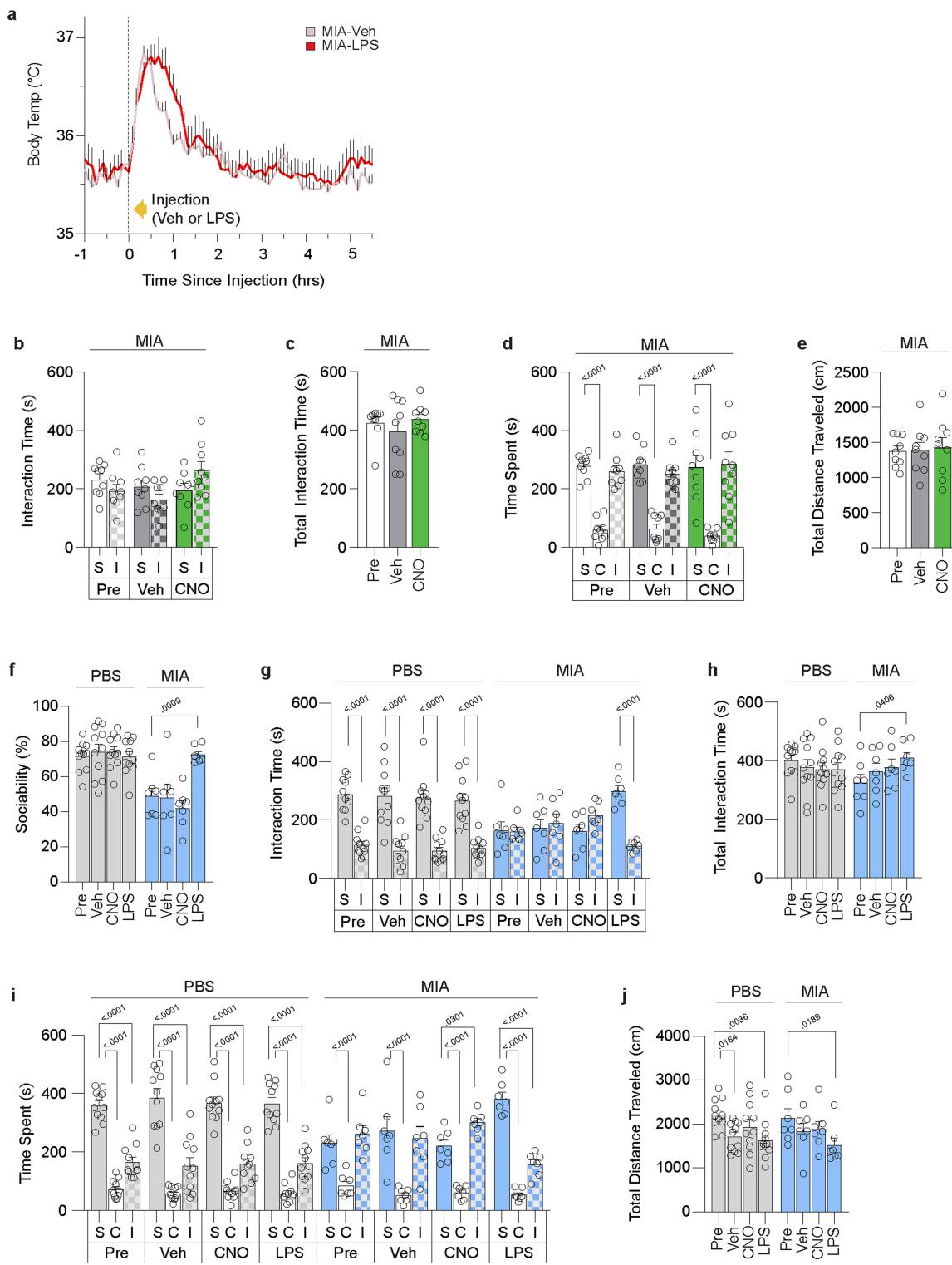
**Extended Data Fig. 2 | Further behavioural analyses for sociability performance after treatment with LPS in PBS and MIA offspring, and monogenic mutant mice.** **a–d**, Time spent investigating social (S) versus inanimate (I) objects (**a**), total interaction time (**b**), time spent in social (S), centre (C) or inanimate (I) chamber (**c**), and distance travelled (**d**) for sociability experiments in Fig. 1c. PBS offspring + vehicle,  $n=10$ ; PBS offspring + LPS,  $n=9$ ; MIA offspring + vehicle,  $n=10$ ; MIA offspring + LPS,  $n=12$ ; wild type (WT) +

vehicle,  $n=8$ ; wild type + LPS,  $n=11$ ; *Cntnap2* mutant + vehicle,  $n=11$ ; *Cntnap2* mutant + LPS,  $n=11$ ; *Fmr1* mutant + vehicle,  $n=11$ ; *Fmr1* mutant + LPS,  $n=15$ ; *Shank3* mutant + vehicle,  $n=8$ ; *Shank3* mutant + LPS,  $n=10$ ; from 3 independent experiments. All  $n$  values refer to the number of mice used. Statistics calculated by two-way ANOVA with Sidak's (**a**) or Dunnett's (**c**) post-hoc test, or two-way repeated-measures ANOVA with Sidak's post-hoc test (**b**, **d**). Graphs are mean  $\pm$  s.e.m.



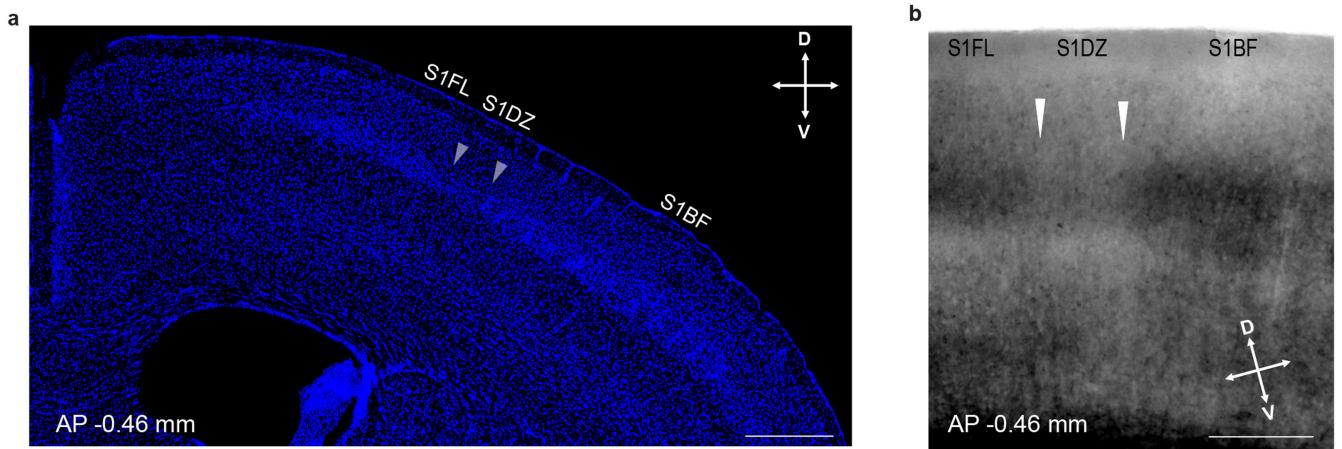
**Extended Data Fig. 3 | LPS-induced rescue of MIA behavioural phenotypes is transient, effective in aged mice and extends beyond three-chambered sociability.** **a–e**, Sociability measured 72 h after injection with vehicle (Veh) or LPS, in PBS and MIA offspring from Fig. 1c. Data expressed as per cent sociability (**a**), time spent investigating social (S) versus inanimate (I) objects (**b**), total interaction time (**c**), time spent in social (S), centre (C) or inanimate (I) chamber (**d**), and distance travelled (**e**) during three-chambered sociability experiments. PBS offspring + vehicle,  $n = 7$ ; PBS offspring + LPS,  $n = 7$ ; MIA offspring + vehicle,  $n = 8$ ; MIA offspring + LPS,  $n = 6$ ; from 2 independent experiments. **f–j**, Sociability measured before and 4 h after injection of vehicle or LPS in aged MIA mice (9–12 months old). Data are expressed as per cent sociability (**f**), time spent investigating social (S) versus inanimate (I) objects (**g**), total interaction time (**h**), time spent in social (S), centre (C) or inanimate (I)

chamber (**i**), and distance travelled (**j**) during three-chambered sociability experiments. MIA offspring + vehicle,  $n = 6$ ; MIA offspring + LPS,  $n = 7$ ; from 2 independent experiments. **k**, Reciprocal social interactions measured after treatment with vehicle or LPS in PBS or MIA offspring. PBS offspring + vehicle,  $n = 9$ ; PBS offspring + LPS,  $n = 9$ ; MIA offspring + vehicle,  $n = 11$ ; MIA offspring + LPS,  $n = 11$ ; from 4 independent experiments. **l**, Marble burying index (percentage of buried marbles) measured before and 4 h after treatment with vehicle or LPS in PBS or MIA offspring. PBS offspring + vehicle,  $n = 12$ ; PBS offspring + LPS,  $n = 12$ ; MIA offspring + vehicle,  $n = 12$ ; MIA offspring + LPS,  $n = 11$ ; from 5 independent experiments. All  $n$  values refer to the number of mice used. Statistics calculated by two-way ANOVA with Sidak's (**a–c, e, g**), Dunnett's (**d, i**) or Tukey's (**k**) post-hoc tests, or two-way repeated-measures ANOVA with Sidak's post-hoc test (**f, h, j, l**). Graphs are mean  $\pm$  s.e.m.



**Extended Data Fig. 4 | Acute increase in body temperature is insufficient to promote sociability.** **a**, Body temperature profile after injection of vehicle or LPS in MIA offspring. Vehicle,  $n=10$ ; LPS,  $n=10$ ; from 4 independent experiments. The initial spike in body temperature is due to handling stress. **b–e**, Data are expressed as time spent investigating social (S) versus inanimate (I) objects (**b**), total interaction time (**c**), time spent in social (S), centre (C) or inanimate (I) chamber (**d**), and distance travelled (**e**) during three-chambered sociability experiments described in Fig. 1g.  $n=9$  for all groups, from 2 independent experiments. **f–j**, Sociability performance in *Vgat*-Cre PBS and MIA offspring after treatment with vehicle, CNO or LPS. Data are expressed as

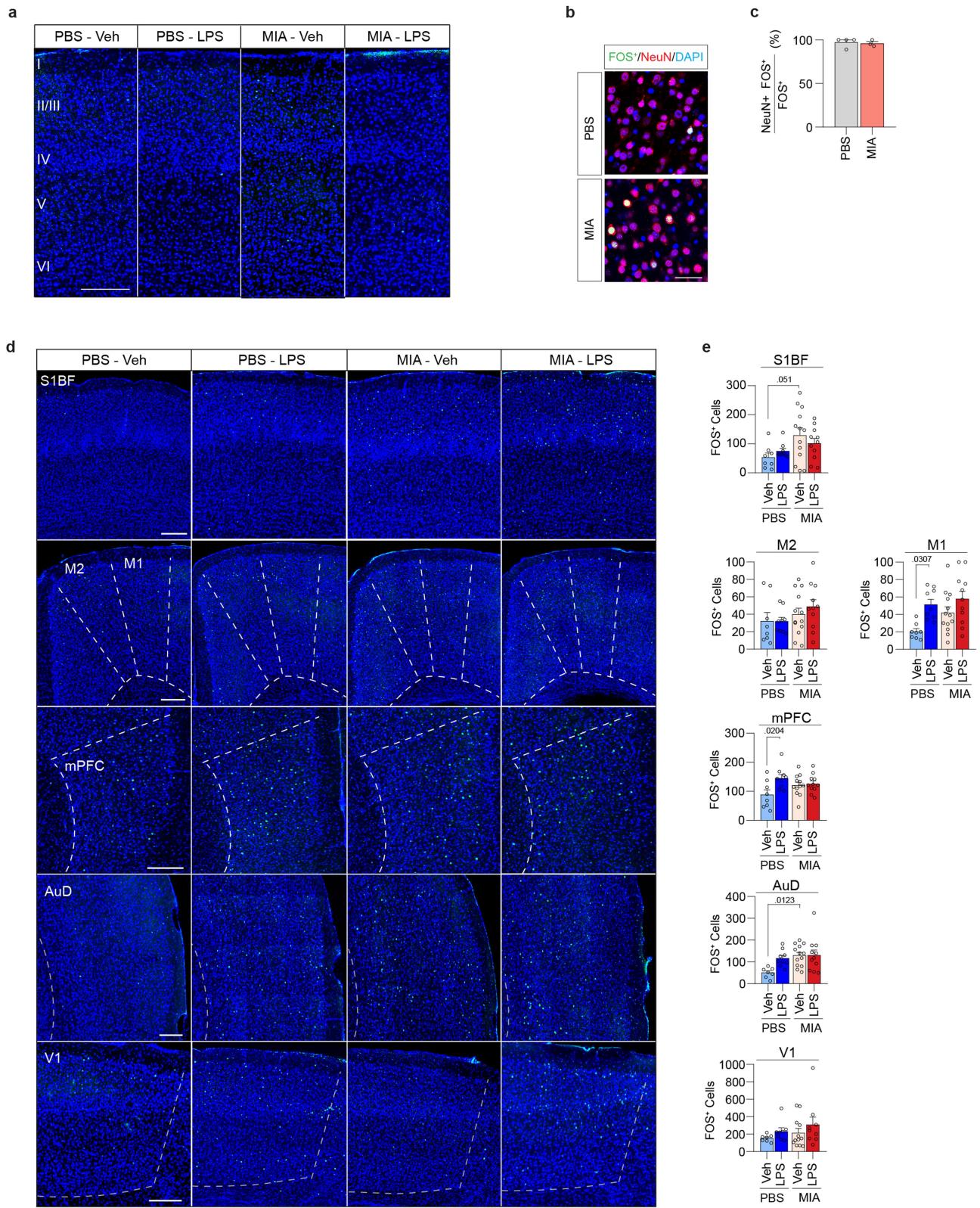
per cent sociability (**f**), time spent investigating social (S) versus inanimate (I) objects (**g**), total interaction time (**h**), time spent in social (S), centre (C) or inanimate (I) chamber (**i**), and distance travelled (**j**) during three-chambered sociability experiments. PBS offspring,  $n=11$ ; MIA offspring,  $n=7$ ; from 2 independent experiments. All  $n$  values refer to the number of mice used. Statistics calculated by two-way repeated-measures ANOVA with Bonferroni's (**a**) or Dunnett's (**f, h, j**) post-hoc tests, two-way ANOVA with Sidak's (**b, g**) or Dunnett's (**d, i**) post-hoc tests, or one-way repeated-measures ANOVA with Tukey's post-hoc test (**c, e**). Graphs are mean  $\pm$  s.e.m.



**Extended Data Fig. 5 | Histological identification of the S1DZ.** **a**, Coronal section of the cortex counterstained with DAPI to highlight the abrupt reduction in cell density in layer 4, between the S1DZ and the S1BF, at AP $-0.46\text{ mm}$ .  $n=5$ , from 1 independent experiment. D, dorsal; V, ventral.

**b**, Coronal section of the cortex imaged with differential interference contrast, further highlighting the reduced layer 4 in the S1DZ at AP $-0.46\text{ mm}$ .  $n=3$ , from 2 independent experiments. All  $n$  values refer to the number of mice used. White arrows indicate borders of S1DZ. Scale bars, 500  $\mu\text{m}$  (**a**); 300  $\mu\text{m}$  (**b**).

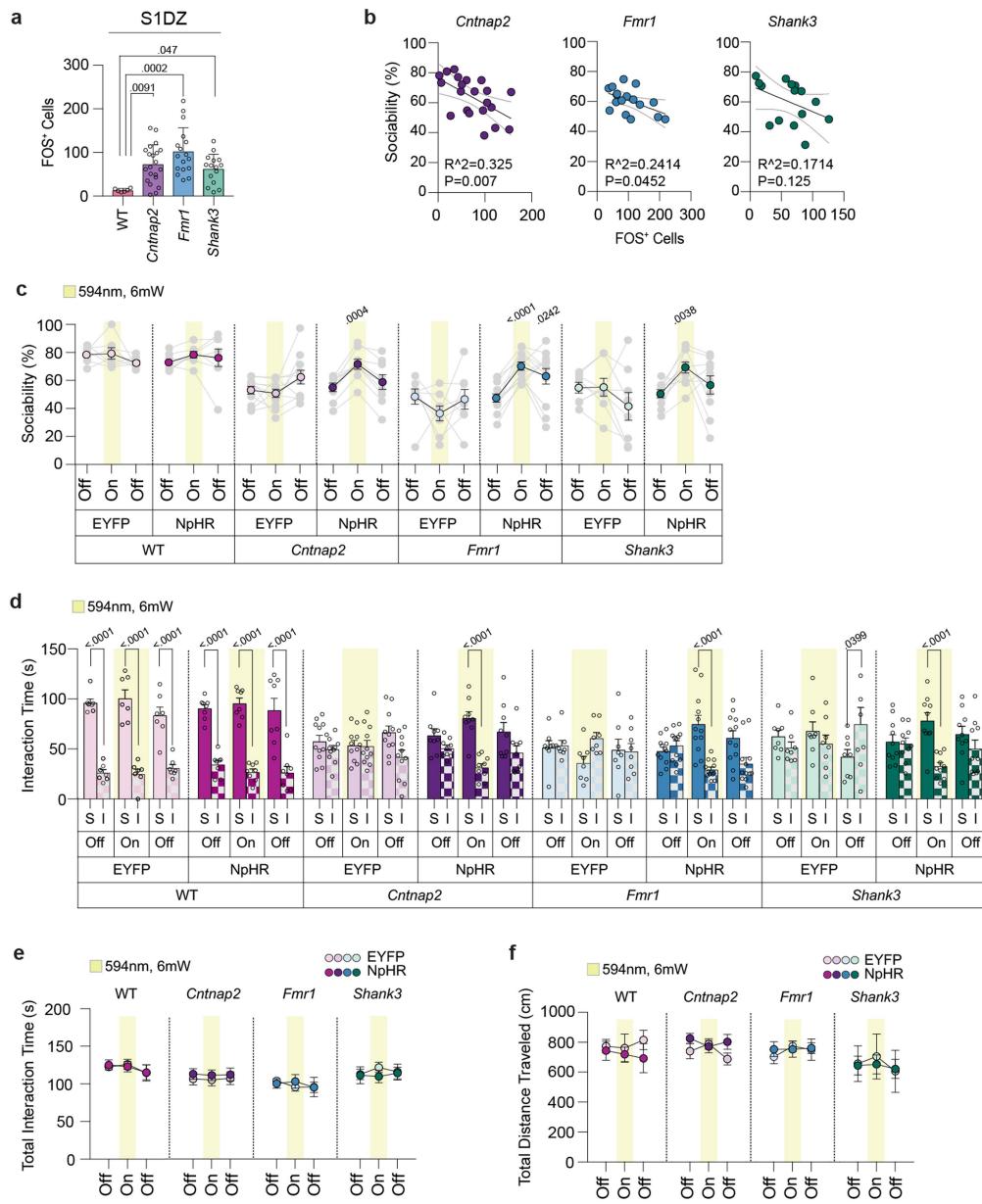
# Article



**Extended Data Fig. 6** | See next page for caption.

**Extended Data Fig. 6 | Treatment of MIA offspring with LPS does not have a distinguishable effect on FOS expression in the other cortical regions analysed.** **a**, Full cortical depth of S1DZ FOS staining as shown in Fig. 2a, for PBS and MIA offspring after administration of vehicle or LPS. PBS offspring + vehicle,  $n = 8$ ; PBS offspring + LPS,  $n = 9$ ; MIA offspring + vehicle,  $n = 13$ ; MIA offspring + LPS,  $n = 11$ ; from 3 independent experiments. Scale bar, 200  $\mu\text{m}$ . **b, c**, Representative images (**b**) and quantification (**c**) of FOS (green) and NeuN (red) colabelled cells within the S1DZ of PBS and MIA offspring. PBS offspring,  $n = 4$ ; MIA offspring,  $n = 3$ ; from 1 independent experiment. Scale bar, 50  $\mu\text{m}$ . **d, e**, Representative images (**d**) and quantification (**e**) of FOS expression in a series of cortical regions and after injection of vehicle or LPS in PBS or MIA

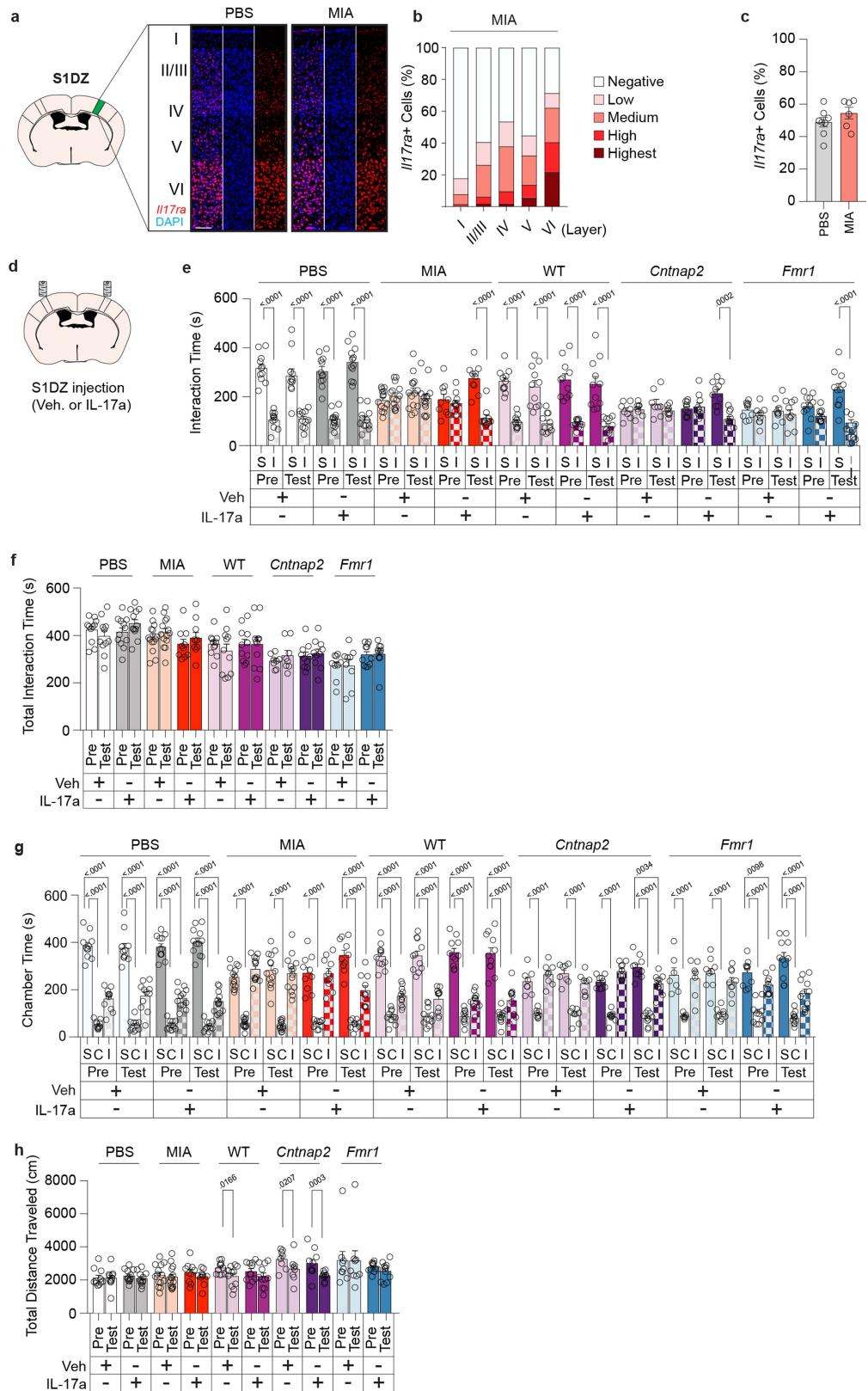
offspring. Sections are stained for FOS (green) and DAPI (blue). Scale bars, 200  $\mu\text{m}$ . For S1BF, M2, M1 and AuD: PBS offspring + vehicle,  $n = 8$ ; PBS offspring + LPS,  $n = 9$ ; MIA offspring + vehicle,  $n = 13$ ; MIA offspring + LPS,  $n = 11$ . For mPFC: PBS offspring + vehicle,  $n = 8$ ; PBS offspring + LPS,  $n = 9$ ; MIA offspring + vehicle,  $n = 12$ ; MIA offspring + LPS,  $n = 11$ . For V1: PBS offspring + vehicle,  $n = 7$ ; PBS offspring + LPS,  $n = 8$ ; MIA offspring + vehicle,  $n = 12$ ; MIA offspring + LPS,  $n = 9$ ; from 4 independent experiments. All  $n$  values refer to the number of mice used. Statistics calculated by unpaired two-tailed *t*-test (**c**) or two-way ANOVA with Tukey's post-hoc test (**e**). Graphs are mean  $\pm$  s.e.m.



#### Extended Data Fig. 7 | Further behavioural analyses of S1DZ optical-inhibition-mediated rescue of sociability in monogenic mutant mice.

**a**, Quantification of FOS-expressing cells in the S1DZ of monogenic mutant mice. Wild type,  $n=6$ ; *Cntnap2* mutant,  $n=21$ ; *Fmr1* mutant,  $n=17$ ; *Shank3* mutant,  $n=15$ ; from 5 independent experiments. **b**, Correlation of FOS expression in the S1DZ with severity of sociability deficits across monogenic mutant mice. *Cntnap* mutant,  $n=21$ ; *Fmr1* mutant,  $n=17$ ; *Shank3* mutant,  $n=15$ ; from 4 independent experiments. Black solid lines represent regression line; grey lines indicate 90% confidence intervals. **c**, Individual data for experiments in Fig. 2f. **d-f**, Data are expressed as time spent investigating social (S) versus

inanimate (I) objects (**d**), total interaction time (**e**), and distance travelled (**f**) during the three-chambered sociability experiments described in Fig. 2f. Wild type + EYFP,  $n=7$ ; wild type + NpHR,  $n=8$ ; *Cntnap2* mutant, + EYFP,  $n=11$ ; *Cntnap2* mutant + NpHR,  $n=9$ ; *Fmr1* mutant + EYFP,  $n=8$ ; *Fmr1* mutant + NpHR,  $n=12$ ; *Shank3* mutant + EYFP,  $n=8$ ; *Shank3* mutant + NpHR,  $n=10$ ; from 6 independent experiments. All  $n$  values refer to the number of mice used. Statistics calculated by one-way ANOVA with Dunnett's post-hoc test (**a**), linear regression (**b**), one-way repeated-measures ANOVA with Dunnett's post-hoc test (**c**), two-way ANOVA with Sidak's post-hoc test (**d**) or two-way repeated-measures ANOVA with Sidak's post-hoc test (**e,f**). Graphs are mean  $\pm$  s.e.m.

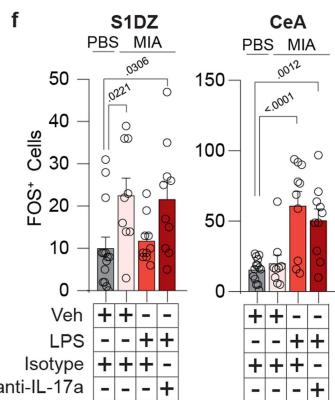
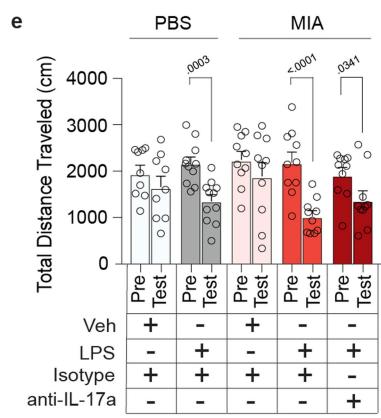
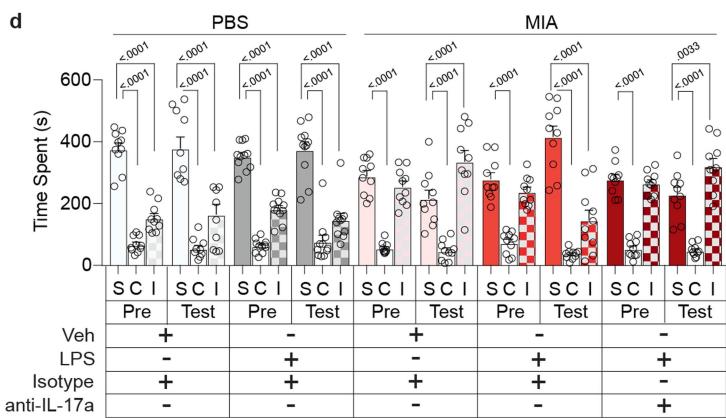
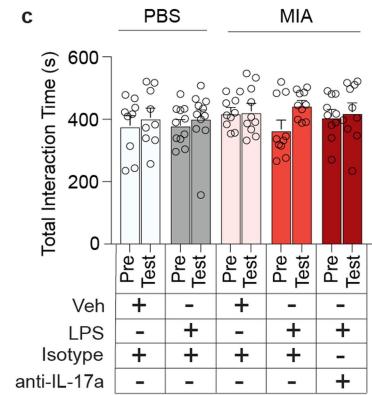
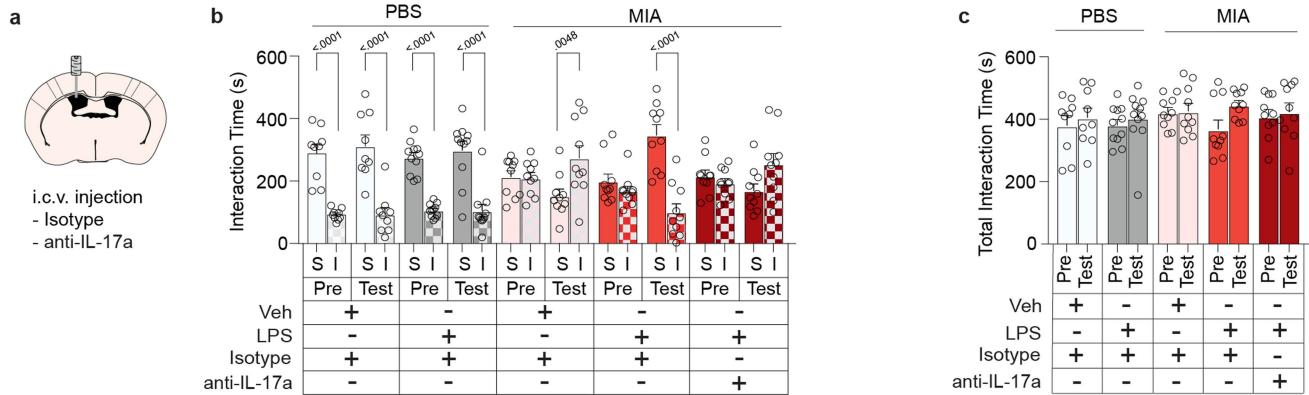


**Extended Data Fig. 8** | See next page for caption.

# Article

**Extended Data Fig. 8 | *Il17ra* expression in the S1DZ of PBS and MIA offspring and further behavioural analyses of S1DZ IL-17a rescue of sociability in MIA Offspring and monogenic mutant mice.** **a**, Representative images of *Il17ra* expression in the S1DZ of PBS and MIA offspring. Scale bar, 1 mm. **b**, Quantification of *Il17ra* expression within the S1DZ of MIA offspring according to cortical layer.  $n = 6$ , from 2 independent experiments. **c**, Quantification of overall *Il17ra* expression in the S1DZ of PBS and MIA offspring. PBS offspring,  $n = 8$ ; MIA offspring,  $n = 6$ ; from 2 independent experiments. **d–h**, Further behavioural analyses of experiments described in Fig. 3f. Time spent investigating social (S) versus inanimate (I) objects (**e**), total

interaction time (**f**), time spent in social (S), centre (C) or inanimate (I) chamber (**g**), and distance travelled (**h**). PBS offspring + vehicle,  $n = 11$ ; PBS offspring + IL-17a,  $n = 12$ ; MIA offspring + vehicle,  $n = 14$ ; MIA offspring + IL-17a,  $n = 10$ ; wild type + vehicle,  $n = 11$ ; wild type + IL-17a,  $n = 11$ ; *Ctnnap2* mutant + vehicle,  $n = 8$ ; *Ctnnap2* mutant + IL-17a,  $n = 10$ ; *Fmr1* mutant + vehicle,  $n = 9$ ; *Fmr1* mutant + IL-17a,  $n = 11$ ; from 6 independent experiments. All  $n$  values refer to the number of mice used. Statistics calculated by unpaired two-tailed *t*-test (**c**), two-way ANOVA with Sidak's (**e**) or Dunnett's (**g**) post-hoc tests, or two-way repeated-measures ANOVA with Sidak's post-hoc test (**f**, **h**). Graphs are mean  $\pm$  s.e.m.

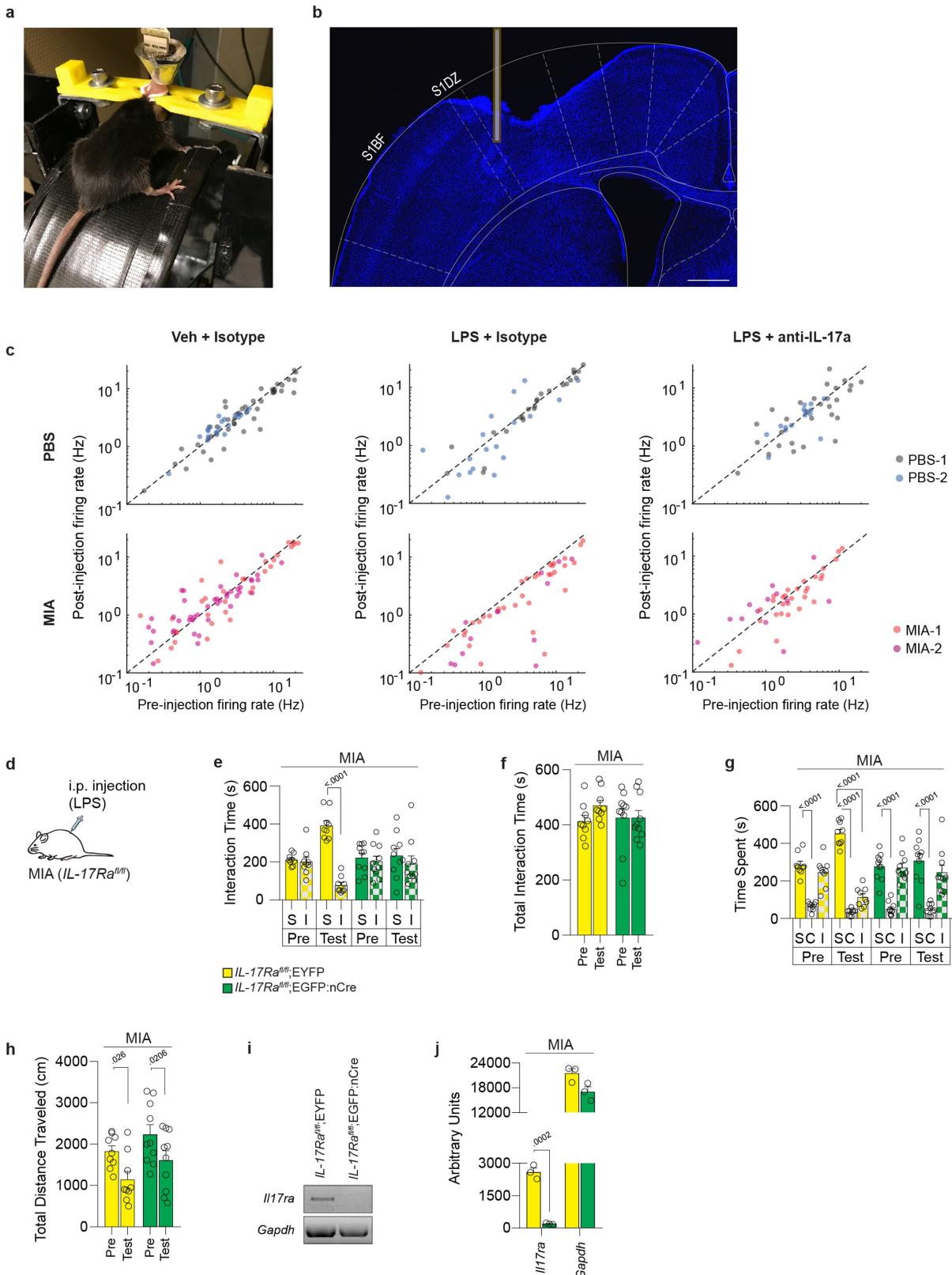


**Extended Data Fig. 9 | IL-17a is necessary for LPS-induced behavioural rescue and reduction of FOS expression in MIA offspring.** **a–e.** Further behavioural analyses of experiments described in Fig. 4a. Time spent investigating social (S) versus inanimate (I) objects (**b**), total interaction time (**c**), time spent in social (S), centre (C) or inanimate (I) chamber (**d**), and distance travelled (**e**). PBS offspring + vehicle + isotype,  $n=9$ ; PBS offspring + LPS + isotype,  $n=11$ ; MIA offspring + vehicle + isotype,  $n=10$ ; MIA offspring + LPS + isotype,  $n=10$ ; MIA offspring + LPS + anti-IL-17a,  $n=10$ ; from 7 independent experiments.

**f.** Quantification of FOS-expressing cells in the S1DZ and CeA after injection of

vehicle or LPS in MIA offspring pre-treated intracerebroventricularly with isotype-control antibody or blocking antibody against IL-17a (anti-IL-17a). PBS offspring + vehicle + isotype,  $n=14$ ; MIA offspring + vehicle + isotype,  $n=9$ ; MIA offspring + LPS + isotype,  $n=10$ ; MIA offspring + LPS + anti-IL-17a,  $n=10$ ; from 4 independent experiments. All  $n$  values refer to the number of mice used. Statistics calculated by two-way ANOVA with Sidak's (**b**) or Dunnett's (**d**) post-hoc tests, two-way repeated-measures ANOVA with Sidak's post-hoc tests (**c**, **e**) or one-way ANOVA with Dunnett's post-hoc test (**f**). Graphs are mean  $\pm$  s.e.m.

# Article



**Extended Data Fig. 10** | See next page for caption.

**Extended Data Fig. 10 | Further analyses of the necessity of IL-17a for the LPS-induced reduction of firing rate in the S1DZ, and the necessity of S1DZ IL-17Ra expression for the LPS-induced rescue of sociability deficits in MIA offspring.** **a–c**, Further analyses for experiments described in Fig. 4b–d. **a**, Example of a head-fixed mouse on the running wheel used during single-unit recording. **b**, Representative image of a tetrode placement in the S1DZ. Scale bar, 500  $\mu$ m. **c**, Firing rate for individual cells before and 4 h after injection of vehicle or LPS in PBS and MIA offspring pre-treated with isotype-control antibody or blocking antibody against IL-17a (anti-IL-17a). **d–h**, Further analyses for experiments described in Fig. 4e, f. Time spent investigating social (S)

versus inanimate (I) objects (e), total interaction time (f), time spent in social (S), centre (C) or inanimate (I) chambers (g), and distance travelled (h). *Il-17ra<sup>f/f</sup>; EYFP*,  $n=9$ ; *Il-17ra<sup>f/f</sup>; EGFP:nCre*,  $n=10$ ; from 5 independent experiments. **i,j**, Representative images (i) and corresponding quantification (j) of *Il17ra* and *Gapdh* amplicon following PCR using cDNA derived from cells isolated from the cortical region centred on S1DZ of *Il-17ra<sup>f/f</sup>; EYFP* and *Il-17ra<sup>f/f</sup>; EGFP:nCre* mice.  $n=3$  for both groups; from 1 experiment. All  $n$  values refer to the number of mice used. Statistics calculated by two-way ANOVA with Sidak's post-hoc test (e,g), two-way repeated-measures ANOVA with Sidak's post-hoc tests, (f,h) or unpaired two-tailed *t*-test (j). Graphs are mean  $\pm$  s.e.m.

Corresponding author(s): Gloria B. Choi

Last updated by author(s): 10/17/2019

# Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided  
*Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g.  $F$ ,  $t$ ,  $r$ ) with confidence intervals, effect sizes, degrees of freedom and  $P$  value noted  
*Give P values as exact values whenever suitable.*
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's  $d$ , Pearson's  $r$ ), indicating how they were calculated

*Our web collection on [statistics for biologists](#) contains articles on many of the points above.*

## Software and code

Policy information about [availability of computer code](#)

Data collection

Behavioral experiments were recorded using Ethovision XT. IL-17R knockout and c-Fos+ cell quantification were performed using FIJI (ImageJ 1.49m). qPCR data for SFB quantification were collected using Lightcycler R 96 SW1.1. In situ and immunohistochemistry automated quantification was performed using QuPath. Single unit recording data were acquired using a Neuralynx multiplexing digital recording system. Spike sorting for single-unit recording data was performed using MountainSort and the MClust toolbox.

Data analysis

Data were analyzed using Graphpad Prism v8.0.1.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

## Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

*Provide your data availability statement here.*

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences       Behavioural & social sciences       Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](http://nature.com/documents/nr-reporting-summary-flat.pdf)

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was chosen based on similar previous studies (8,15)
Data exclusions	Behavioral results from mice with inaccurate targeting of viral infection or cannula implantations were excluded.
Replication	Key experiments were reiterated with representatives from each group with similar observations across iterations.
Randomization	Within each iteration of an experiment, animals were randomly assigned to groups with approximately balanced sample size.
Blinding	Experimenters were blind to subject treatment during data collection and analysis.

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input type="checkbox"/>	<input checked="" type="checkbox"/> Clinical data

### Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Antibodies

### Antibodies used

chicken anti-GFP (1:1000, Ab5450, Abcam), rabbit anti-cFos (1:500, ABE457, Millipore), rabbit anti-DsRed (1:1000, 632496, Clontech), mouse anti-NeuN (1:1000, MAB377, Millipore).

### Validation

These are all commonly used antibodies. Statements regarding validation can be found at the manufacturers website.

## Animals and other organisms

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	WT mice used for experiments were male C57BL/6 mice. Male IL17Ra KO, IL17Ra(fl/fl), Cntnap2, Fmr1, Shank3, and Vgat-Cre mice in the C57BL/6 background were also used for experiments. Sources for all mice used are reported in the methods.
Wild animals	Study did not involve wild animals.
Field-collected samples	Study did not involve field-collected samples
Ethics oversight	Committee on Animal Care at Massachusetts Institute of Technology provided approval for all experiments.

Note that full information on the approval of the study protocol must also be provided in the manuscript.